

Mutant-specific oligonucleotide primers used for mutant number 1. Mutated nucleotide underlined.

*Bet v 1* sense            5'- AATTATGAGACTGAGACCCCCTCTGTTATCCCAGCAGCTCG -3'

*Bet v 1* non-sense        3'- TTAATACTCTGACTCTGGTGGAGACAATAGGGTCGTCGAGC -5'

sense primer            5'-                            TGAGACCCCCTCTGTTATCCCAG                            -3'

non-sense primer        3'-                            ATACTCTGACTCTGGGGGAGACA                            -5'

Fig. 2

Oligonucleotide primers for site directed mutagenesis of  
Bet v 1 (No. 2801).

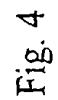
all	sense	1: 183Bv, 15-mer 5'-GTTGCCAACGATCAG
1	sense	2: 184Bv, 23-mer 5'-TGAGACCCCCTCTGTATCCCAG
1	non-sense	3: 185Bv, 23-mer 5'-ACAGAGGGGTCTCAGTCTCATA
2	sense	4: 186Bv, 31-mer 5'-GATACCTCTTTCCACAGTTGCACCCCAAG
2	non-sense	5: 187Bv, 31-mer 5'-ACCTGTGGAAAGAGGGTATCGCCATCAAGGA
3	sense	6: 188Bv, 23-mer 5'-AACATTTTCAGGAAATGGAGGGCC
3	non-sense	7: 189Bv, 23-mer 5'-TTTCCTGAAATGTTTCAACACT
4	sense	8: 190Bv, 23-mer 5'-TTAAGAACATCAGCTTTCCCGAA
4	non-sense	9: 191Bv, 23-mer 5'-AGCTGATGTTCTTAATGGTTCCA
5	sense	10: 192Bv, 23-mer 5'-GGACCATGCAAACTTCAAATACA
5	non-sense	11: 193Bv, 23-mer 5'-AGTTTGCATGGTCCACCTCATCA
6	sense	12: 194Bv, 23-mer 5'-TTTCCTCAGGCCTCCCTTTCAA
6	non-sense	13: 195Bv, 23-mer 5'-AGGCCTGAGGGAAAGCTGATCTT
7	sense	14: 196Bv, 24-mer 5'-TGAAGGATCTGGAGGGCCTGGAAC
7	non-sense	15: 197Bv, 24-mer 5'-CCCTCCAGATCCTTCAATGTTTTC
8	sense	16: 198Bv, 24-mer 5'-GGCAACTGGTGATGGAGGATCCAT
8	non-sense	17: 199Bv, 24-mer 5'-CCATCACCAGTTGCCACTATCTTT
all	non-sense	18: 200Bv, 15-mer 5'-CATGCCATCCGTAAG

10001245.333504

Fig. 3

## Overview of all Bet v 1 mutations

1 (A-C)	
GGTGTGTTTAATTATGAGACTGAGACCACTCTGTTATCCCAGCAGCTCGACTGTTCAAG	60
G V F N Y E T E T T P S V I P A A R L F K	20
9 (A-G) 2 (A-C) 2 (A-C)	
GCCTTTATCCTTGATGGGATAAAGGTTGCACCCCAAGCCATTAGCAGT	120
A F I L D-G G D N-T L F P K-Q V A P Q A I S S	40
3 (GA-TC) 7 (AA-TC) 4 (G-C) 6 (GA-TC)	
GTTGAAAACATTGAAGGAAATGGAGGGCCTGGAACCATTAAGAAGATCAGCTTTCCCGAA	180
V E N I E-S G N-S G G P G T I K K-N I S F P E-S	60
5 (CA-TG)	
GGCCTCCCTTTCAAGTACGTGAAGGACAGAGTTGATGAGGTGGACCAACAACCTTCAAA	240
G L P F K Y V K D R V D E V D H T-A N F K	80
TACAATTACAGCGTGATCGAGGGCGGTCCCATAGGCGACACATTGGAGAAGATCTCCAAC	300
Y N Y S V I E G G P I G D T L E K I S N	100
10 (GAG-CAC) 8 (CCC-TGG)	
GAGATAAAGATAGTGGCAACCCCTGATGGAGGATCCATCTTGAAGATCAGCAACAAGTAC	360
E I K I V A T P-G D G G S I L K I S N K Y	120
CACACCAAAGGTGACCATGAGGTGAAGGCAGAGCAGGTTAAGGCAAGTAAAGAAATGGGC	420
H T K G D H E V K A E Q V K A S K E M G	140
GAGACACTTTTGAGGGCCGTTGAGAGCTACCTCTTGGCACACTCCGATGCCTACAACATAA	480
E T L L R A V E S Y L L A H S D A Y N stop	159



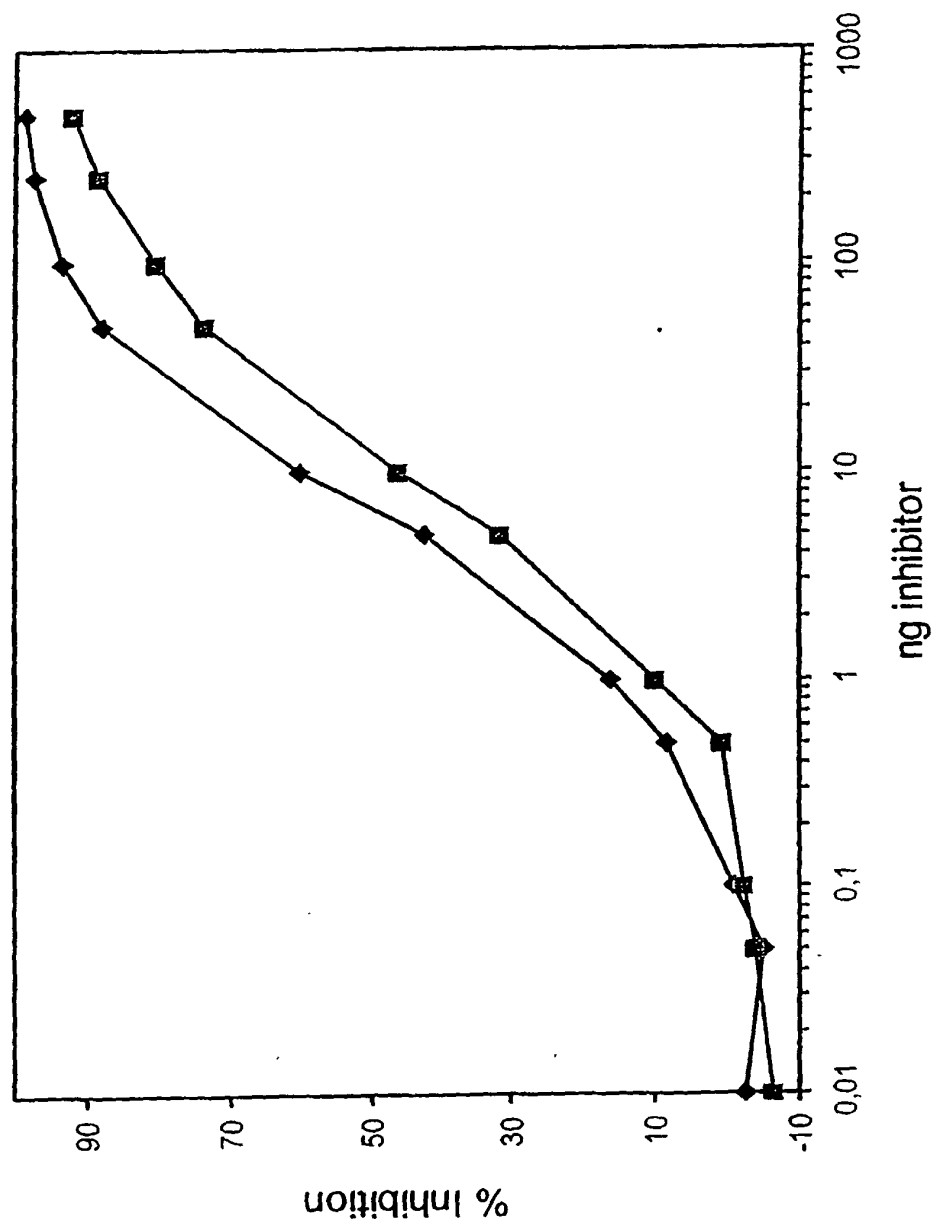


Fig. 5 — Bet v 1 — Asn28Thr+Lys32Gln

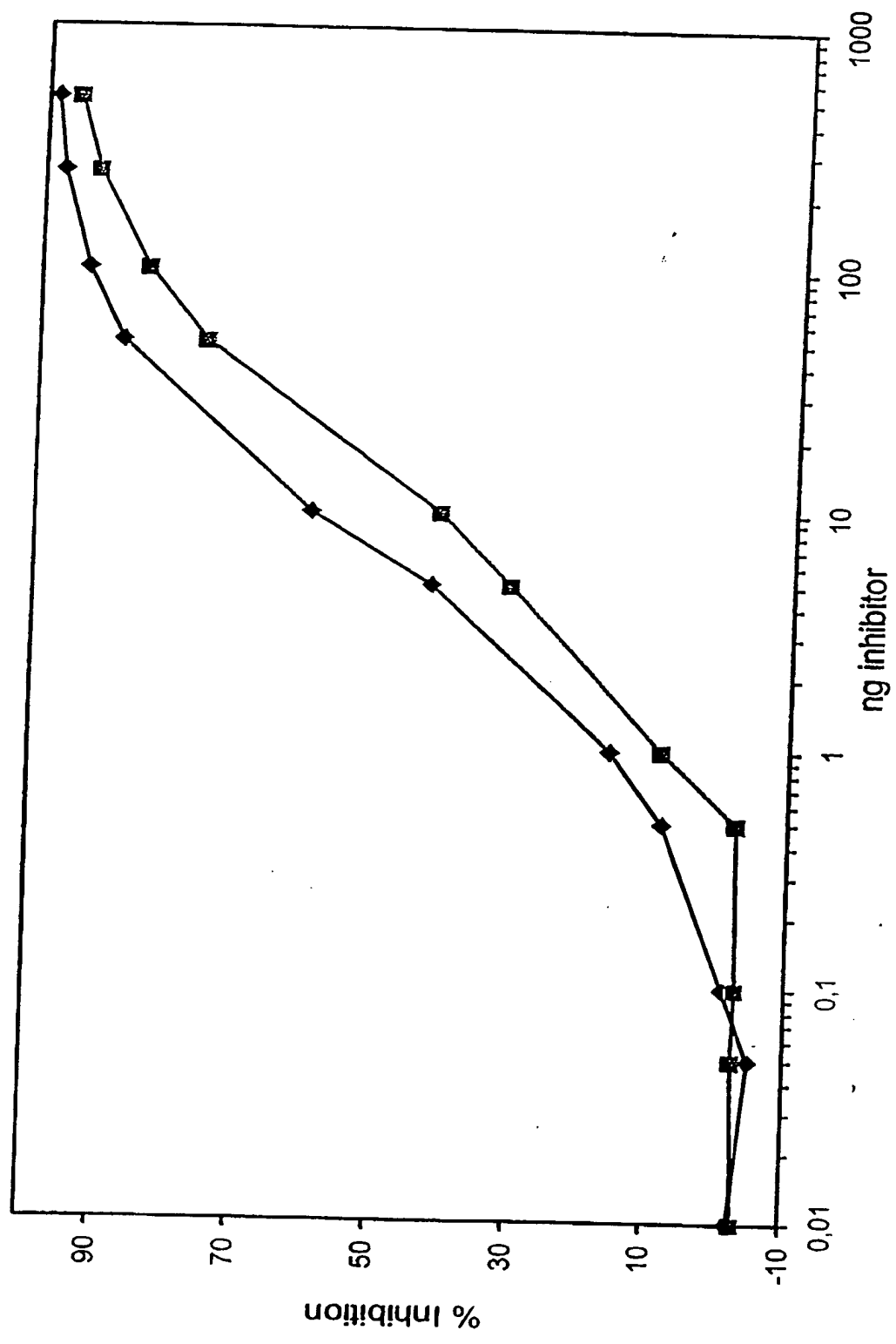


Fig. 6  
—◆— Bet v 1 —■— Pro108Gly

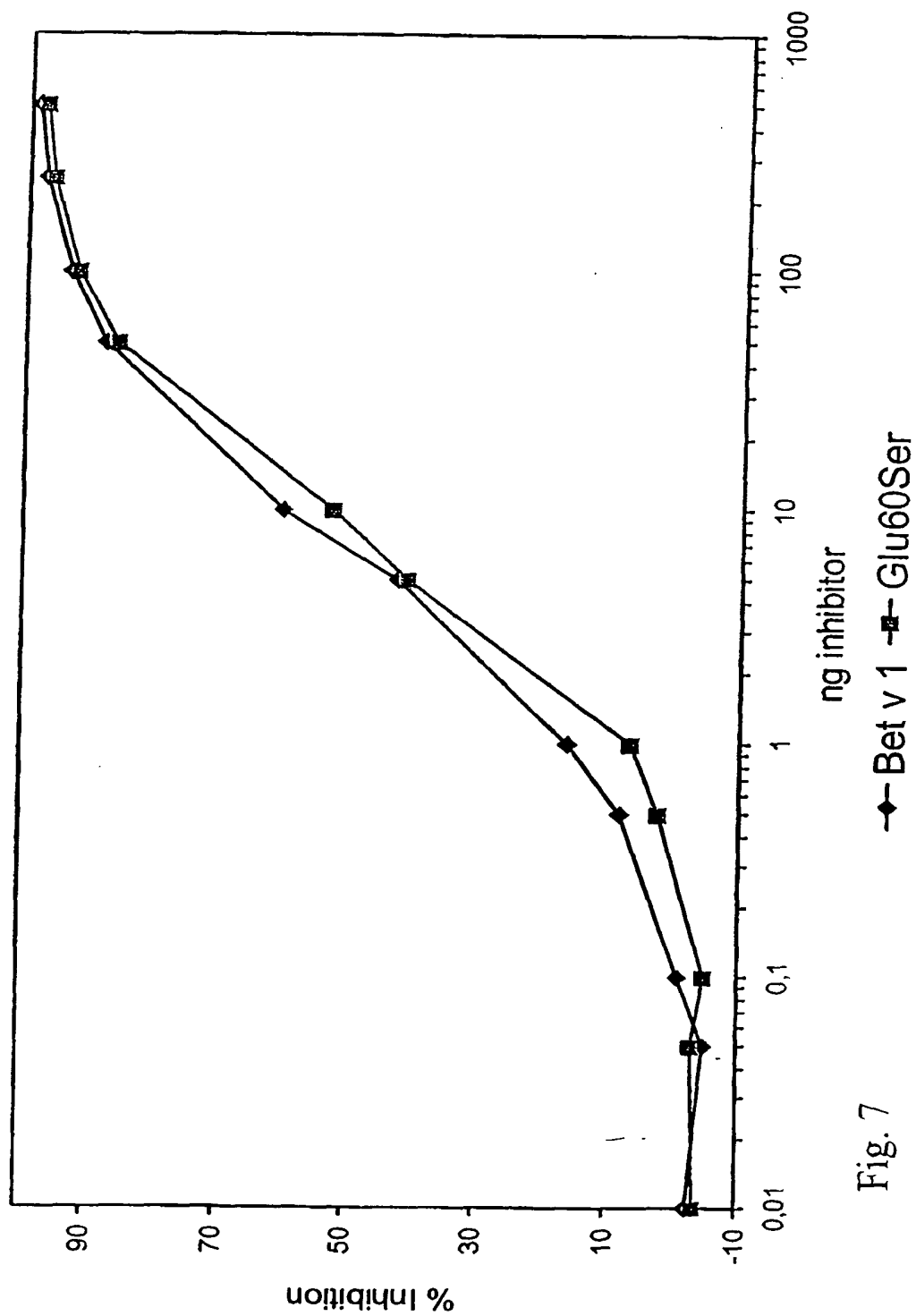


Fig. 7

705111 5427000  
 10mM Na2HPO4/NaH2PO4 0.02MNa3N3

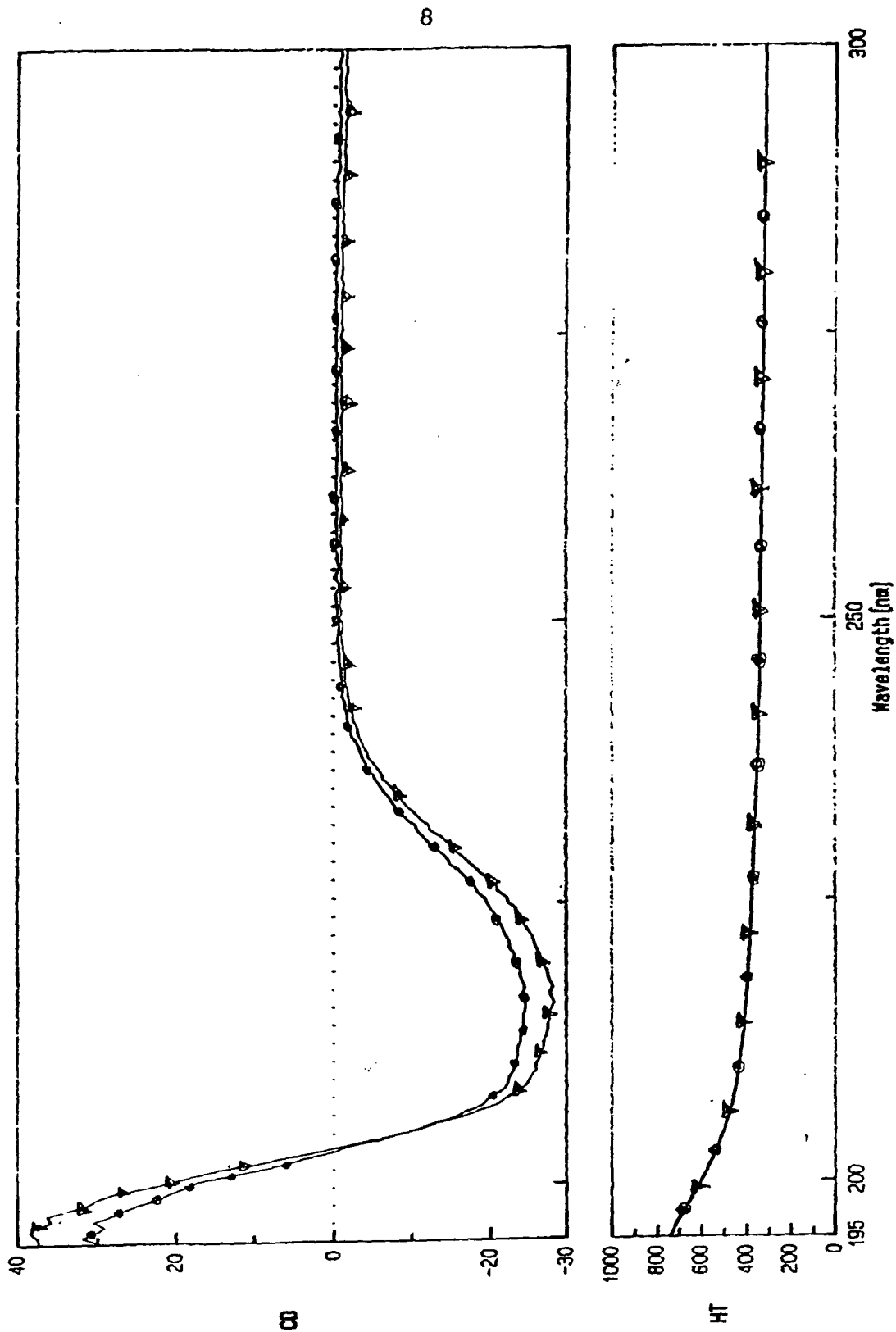


FIG. 8

—△— 5798a r Batv 2801  
 - -●- - 5797a 3Mutant 2595



10511 5425003

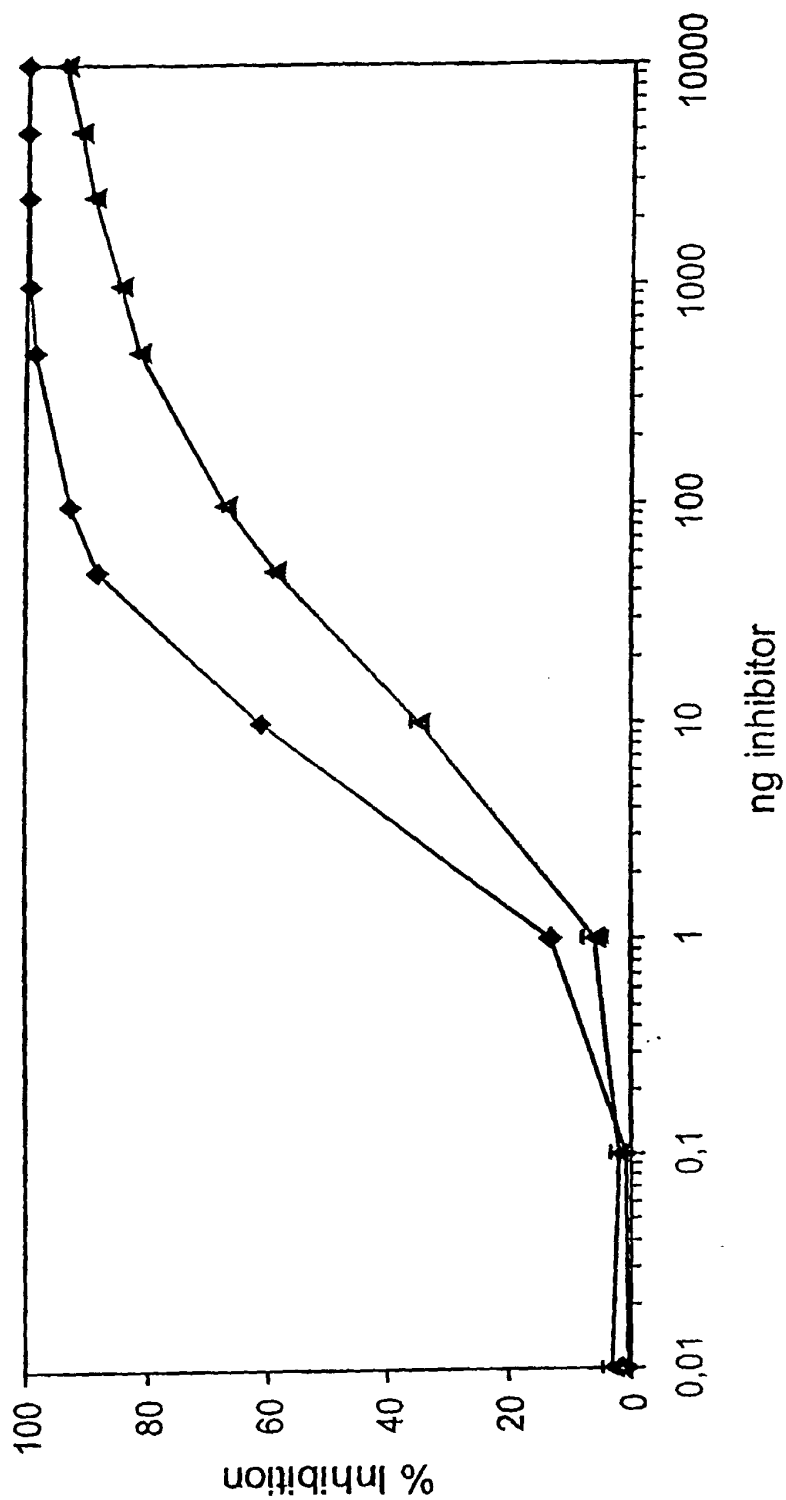
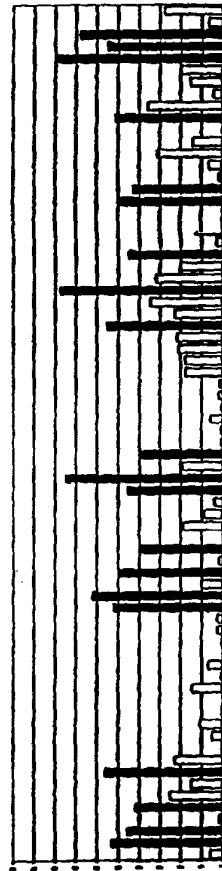
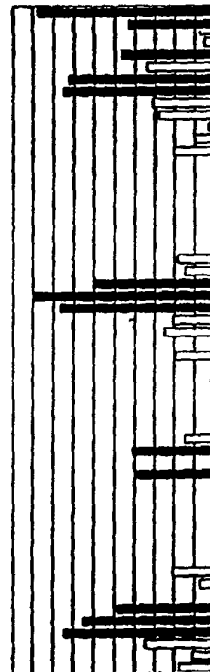


Fig. 9

The image displays a large, intricate grid of characters, primarily uppercase letters, arranged in a dense, rectangular pattern. The grid is composed of many small squares, each containing a single character. The characters are a mix of uppercase and lowercase letters, numbers, and symbols. The grid is arranged in a roughly rectangular shape, with some areas appearing more densely packed than others. The overall appearance is that of a highly structured, repetitive pattern.



22 23 24 25 26 27 28 29 30 31 32 33 34 35 36 37 38 39 40 41 42 43 44 45 46 47 48 49 50 51 52 53 54 55 56 57 58 59 60 61 62 63 64 65 66 67 68 69 70 71 72 73 74 75 76 77 78 79 80 81 82 83 84 85 86 87 88 89 90 91 92 93 94 95 96 97 98 99 100 101 102 103 104 105 106 107 108 109 110 111 112 113 114 115 116 117 118 119 120 121 122 123 124 125 126 127 128 129 130 131 132 133 134 135 136 137 138 139 140 141 142 143 144 145 146 147 148 149 150 151 152 153 154 155 156 157 158 159 160 161 162 163 164 165 166 167 168 169 170 171 172 173 174 175 176 177 178 179 180 181 182 183 184 185 186 187 188 189 190 191 192 193 194 195 196 197 198 199 200 201 202 203 204 205 206 207 208 209 210 211 212 213 214 215 216 217 218 219 220 221 222 223 224 225 226 227 228 229 230 231 232 233 234 235 236 237 238 239 240 241 242 243 244 245 246 247 248 249 250 251 252 253 254 255 256 257 258 259 260 261 262 263 264 265 266 267 268 269 270 271 272 273 274 275 276 277 278 279 280 281 282 283 284 285 286 287 288 289 290 291 292 293 294 295 296 297 298 299 300 301 302 303 304 305 306 307 308 309 310 311 312 313 314 315 316 317 318 319 320 321 322 323 324 325 326 327 328 329 330 331 332 333 334 335 336 337 338 339 340 341 342 343 344 345 346 347 348 349 350 351 352 353 354 355 356 357 358 359 360 361 362 363 364 365 366 367 368 369 370 371 372 373 374 375 376 377 378 379 380 381 382 383 384 385 386 387 388 389 390 391 392 393 394 395 396 397 398 399 400 401 402 403 404 405 406 407 408 409 410 411 412 413 414 415 416 417 418 419 420 421 422 423 424 425 426 427 428 429 430 431 432 433 434 435 436 437 438 439 440 441 442 443 444 445 446 447 448 449 450 451 452 453 454 455 456 457 458 459 460 461 462 463 464 465 466 467 468 469 470 471 472 473 474 475 476 477 478 479 480 481 482 483 484 485 486 487 488 489 490 491 492 493 494 495 496 497 498 499 500 501 502 503 504 505 506 507 508 509 510 511 512 513 514 515 516 517 518 519 520 521 522 523 524 525 526 527 528 529 530 531 532 533 534 535 536 537 538 539 540 541 542 543 544 545 546 547 548 549 550 551 552 553 554 555 556 557 558 559 560 561 562 563 564 565 566 567 568 569 570 571 572 573 574 575 576 577 578 579 580 581 582 583 584 585 586 587 588 589 590 591 592 593 594 595 596 597 598 599 600 601 602 603 604 605 606 607 608 609 610 611 612 613 614 615 616 617 618 619 620 621 622 623 624 625 626 627 628 629 630 631 632 633 634 635 636 637 638 639 640 641 642 643 644 645 646 647 648 649 650 651 652 653 654 655 656 657 658 659 660 661 662 663 664 665 666 667 668 669 670 671 672 673 674 675 676 677 678 679 680 681 682 683 684 685 686 687 688 689 690 691 692 693 694 695 696 697 698 699 700 701 702 703 704 705 706 707 708 709 710 711 712 713 714 715 716 717 718 719 720 721 722 723 724 725 726 727 728 729 730 731 732 733 734 735 736 737 738 739 740 741 742 743 744 745 746 747 748 749 750 751 752 753 754 755 756 757 758 759 760 761 762 763 764 765 766 767 768 769 770 771 772 773 774 775 776 777 778 779 780 781 782 783 784 785 786 787 788 789 790 791 792 793 794 795 796 797 798 799 800 801 802 803 804 805 806 807 808 809 810 811 812 813 814 815 816 817 818 819 820 821 822 823 824 825 826 827 828 829 830 831 832 833 834 835 836 837 838 839 840 841 842 843 844 845 846 847 848 849 850 851 852 853 854 855 856 857 858 859 860 861 862 863 864 865 866 867 868 869 870 871 872 873 874 875 876 877 878 879 880 881 882 883 884 885 886 887 888 889 890 891 892 893 894 895 896 897 898 899 900 901 902 903 904 905 906 907 908 909 910 911 912 913 914 915 916 917 918 919 920 921 922 923 924 925 926 927 928 929 930 931 932 933 934 935 936 937 938 939 940 941 942 943 944 945 946 947 948 949 950 951 952 953 954 955 956 957 958 959 960 961 962 963 964 965 966 967 968 969 970 971 972 973 974 975 976 977 978 979 980 981 982 983 984 985 986 987 988 989 990 991 992 993 994 995 996 997 998 999 1000 1001 1002 1003 1004 1005 1006 1007 1008 1009 1010 1011 1012 1013 1014 1015 1016 1017 1018 1019 1020 1021 1022 1023 1024 1025 1026 1027 1028 1029 1030 1031 1032 1033 1034 1035 1036 1037 1038 1039 1040 1041 1042 1043 1044 1045 1046 1047 1048 1049 1050 10



A large, dense grid of small, stylized letters and symbols, resembling a complex code or a highly textured surface. The characters are arranged in a regular pattern, with some larger, more prominent characters interspersed among the smaller ones. The overall effect is one of a highly structured, yet chaotic, visual field.

Figure 10.

Mutant-specific oligonucleotide primers used for Ves v 5 mutants.  
Mutated nucleotides underlined.

Ves v 5 sense 5'- ACCACAGCCTCCAGCGAAGAATATGAAAAATTTGGTATGGA -3'

Ves v 5 non-sense 3'- TGGTGTCTGGAGGTCGCTTCTTATACTTTTTAAACCATACCT -5'

sense primer 5'- CCAGCGCTAATATGAAAAAT -3'

non-sense primer 3'- GTCGGAGGTCGCGGATTATAC -5'

Ves v 5 sense 5'- GGCTAATCAATGTCAATATGGTCACGATACTTGCAGGGGATG -3'

Ves v 5 non-sense 3'- CCGATTAGTTACAGTTATACCAGTGCTATGAACGTCCCTAC -5'

sense primer 5'- TGTCAAAGCTGGTCACGATACT -3'

non-sense primer 3'- TTAGTTACAGTTCCGACCAGTG -5'

12

all sense 1: XhoI start, 38-mer:

EcoRI  
5'-CCGCTCGAGAAAAGAAACAATTATTGTAAATAAAATG  
L E K R N N Y C K I K  
Kex2 cleavage site amino terminus of Ves v 5

1	sense	1: K72As	21-mer	5'-CCAGCGGCTAATATGAAAAAT
1	non-sense	2: K72Aa	21-mer	5'-CATATTAGCCGCTGGAGGCTG
2	sense	3: Y96As	21-mer	5'-TGTC AAGCTGGTCACGATACT
2	non-sense	4: Y96Aa	21-mer	5'-GTGACCAGCTTGACATTGATT
↓				
all	non-sense	7: CT-pPICZαA,	21-mer	5'-ATTCATCAGCTGCGAGATAGG

27710 27711 27712 27713 27714 27715 27716 27717 27718 27719 27720 27721 27722 27723 27724 27725 27726 27727 27728 27729 27730 27731 27732 27733 27734 27735 27736 27737 27738 27739 27740 27741 27742 27743 27744 27745 27746 27747 27748 27749 27750 27751 27752 27753 27754 27755 27756 27757 27758 27759 27760 27761 27762 27763 27764 27765 27766 27767 27768 27769 27770 27771 27772 27773 27774 27775 27776 27777 27778 27779 27780 27781 27782 27783 27784 27785 27786 27787 27788 27789 27790 27791 27792 27793 27794 27795 27796 27797 27798 27799 27800 27801 27802 27803 27804 27805 27806 27807 27808 27809 27810 27811 27812 27813 27814 27815 27816 27817 27818 27819 27820 27821 27822 27823 27824 27825 27826 27827 27828 27829 27830 27831 27832 27833 27834 27835 27836 27837 27838 27839 27840 27841 27842 27843 27844 27845 27846 27847 27848 27849 27850 27851 27852 27853 27854 27855 27856 27857 27858 27859 27860 27861 27862 27863 27864 27865 27866 27867 27868 27869 27870 27871 27872 27873 27874 27875 27876 27877 27878 27879 27880 27881 27882 27883 27884 27885 27886 27887 27888 27889 27890 27891 27892 27893 27894 27895 27896 27897 27898 27899 27900 27901 27902 27903 27904 27905 27906 27907 27908 27909 27910 27911 27912 27913 27914 27915 27916 27917 27918 27919 27920 27921 27922 27923 27924 27925 27926 27927 27928 27929 27930 27931 27932 27933 27934 27935 27936 27937 27938 27939 27940 27941 27942 27943 27944 27945 27946 27947 27948 27949 27950 27951 27952 27953 27954 27955 27956 27957 27958 27959 27960 27961 27962 27963 27964 27965 27966 27967 27968 27969 27970 27971 27972 27973 27974 27975 27976 27977 27978 27979 27980 27981 27982 27983 27984 27985 27986 27987 27988 27989 27990 27991 27992 27993 27994 27995 27996 27997 27998 27999 28000 28001 28002 28003 28004 28005 28006 28007 28008 28009 28010 28011 28012 28013 28014 28015 28016 28017 28018 28019 28020 28021 28022 28023 28024 28025 28026 28027 28028 28029 28030 28031 28032 28033 28034 28035 28036 28037 28038 28039 28040 28041 28042 28043 28044 28045 28046 28047 28048 28049 28050 28051 28052 28053 28054 28055 28056 28057 28058 28059 28060 28061 28062 28063 28064 28065 28066 28067 28068 28069 28070 28071 28072 28073 28074 28075 28076 28077 28078 28079 28080 28081 28082 28083 28084 28085 28086 28087 28088 28089 28090 28091 28092 28093 28094 28095 28096 28097 28098 28099 28100 28101 28102 28103 28104 28105 28106 28107 28108 28109 28110 28111 28112 28113 28114 28115 28116 28117 28118 28119 28120 28121 28122 28123 28124 28125 28126 28127 28128 28129 28130 28131 28132 28133 28134 28135 28136 28137 28138 28139 28140 28141 28142 28143 28144 28145 28146 28147 28148 28149 28150 28151 28152 28153 28154 28155 28156 28157 28158 28159 28160 28161 28162 28163 28164 28165 28166 28167 28168 28169 28170 28171 28172 28173 28174 28175 28176 28177 28178 28179 28180 28181 28182 28183 28184 28185 28186 28187 28188 28189 28190 28191 28192 28193 28194 28195 28196 28197 28198 28199 28200 28201 28202 28203 28204 28205 28206 28207 28208 28209 28210 28211 28212 28213 28214 28215 28216 28217 28218 28219 28220 28221 28222 28223 28224 28225 28226 28227 28228 28229 28230 28231 28232 28233 28234 28235 28236 28237 28238 28239 28240 28241 28242 28243 28244 28245 28246 28247 28248 28249 28250 28251 28252 28253 28254 28255 28256 28257 28258 28259 28260 28261 28262 28263 28264 28265 28266 28267 28268 28269 28270 28271 28272 28273 28274 28275 28276 28277 28278 28279 28280 28281 28282 28283 28284 28285 28286 28287 28288 28289 28290 28291 28292 28293 28294 28295 28296 28297 28298 28299 28300 28301 28302 28303 28304 28305 28306 28307 28308 28309 28310 28311 28312 28313 28314 28315 28316 28317 28318 28319 28320 28321 28322 28323 28324 28325 28326 28327 28328 28329 28330 28331 28332 28333 28334 28335 28336 28337 28338 28339 28340 28341 28342 28343 28344 28345 28346 28347 28348 28349 28350 28351 28352 28353 28354 28355 28356 28357 28358 28359 28360 28361 28362 28363 28364 28365 28366 28367 28368 28369 28370 28371 28372 28373 28374 28375 28376 28377 28378 28379 28380 28381 28382 28383 28384 28385 28386 28387 28388 28389 28390 28391

Fig. 13

13

## Overview of Ves v 5 mutations

1	AACAATTATTGTAAAATAAAATGTTTGAAAGGAGGTGTCCATACTGCCTGCAAATATGGA	60
1	N N Y C K I K C L K G G V H T A C K Y G	20
61	AGTCTTAAACCGAATTGCGGTAATAAGGTAGTGGTATCCTATGGTCTAACGAAACAAGAG	120
21	S L K P N C G N K V V V S Y G L T K Q E	40
121	AAACAAGACATCTTAAAGGAGCACAATGACTTTAGACAAAAAATTGCACGAGGATTGGAG	180
41	K Q D I L K E H N D F R Q K I A R G L E	60
	1 [K72A] (AAG-GCT)	
181	ACTAGAGGTAATCCTGGACCACAGCCTCCAGCGAAGAATATGAAAAATTGGTATGGAAC	240
61	T R G N P G P Q P P A K N M K N L V W N	80
	2 [Y96A] (TA-GC)	
241	GACGAGTTAGCTTATGTGCGCCCAAGTGTGGGCTAATCAATGTCAATATGGTCACGATACT	300
81	D E L A Y V A Q V W A N Q C Q Y G H D T	100
301	TGCAGGGATGTAGCAAAATATCAGGTTGGACAAAACGTAGCCTTAACAGGTAGCACGGCT	360
101	C R D V A K Y Q V G Q N V A L T G S T A	120
361	GCTAAATACGATGATCCAGTTAACTAGTTAAATGTGGGAAGATGAAGTGAAAGATTAT	420
121	A K Y D D P V K L V K M W E D E V K D Y	140
421	AATCCTAAGAAAAAGTTTTTCGGGAAACGACTTTCTGAAAACCGGCCATTACACTCAAATG	480
141	N P K K K F S G N D F L K T G H Y T Q M	160
481	GTTTGGGCTAACACCAAGGAAGTTGGTTGTGGAAGTATAAAATACATTCAAGAGAAATGG	540
161	V W A N T K E V G C G S I K Y I Q E K W	180
541	CACAAACATTACCTTGTATGTAATTATGGACCCAGCGGAAACTTTAAGAATGAGGAACTT	600
181	H K H Y L V C N Y G P S G N F K N E E L	200
601	TATCAAACAAAGTAA	612
201	Y Q T K stop	204

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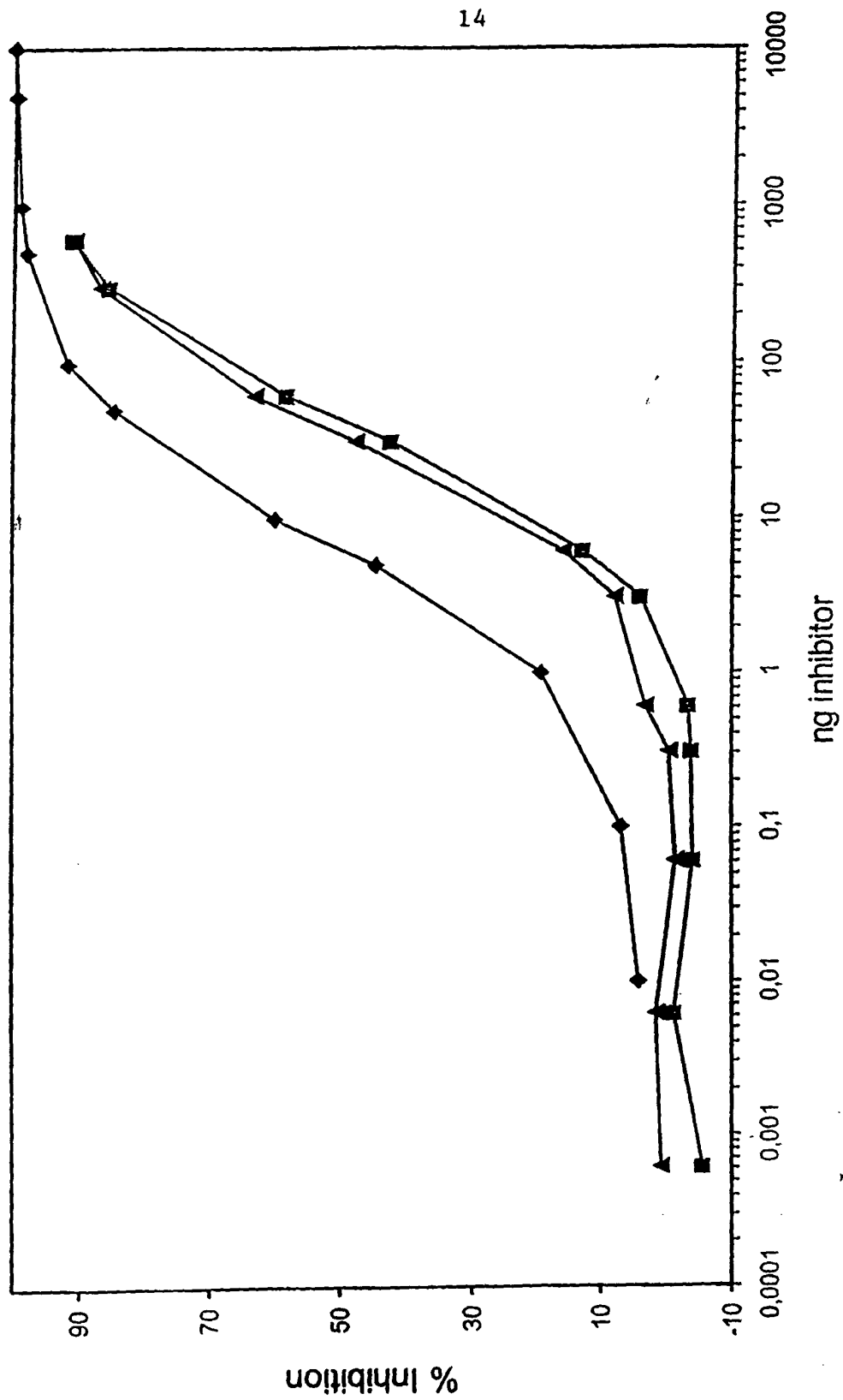
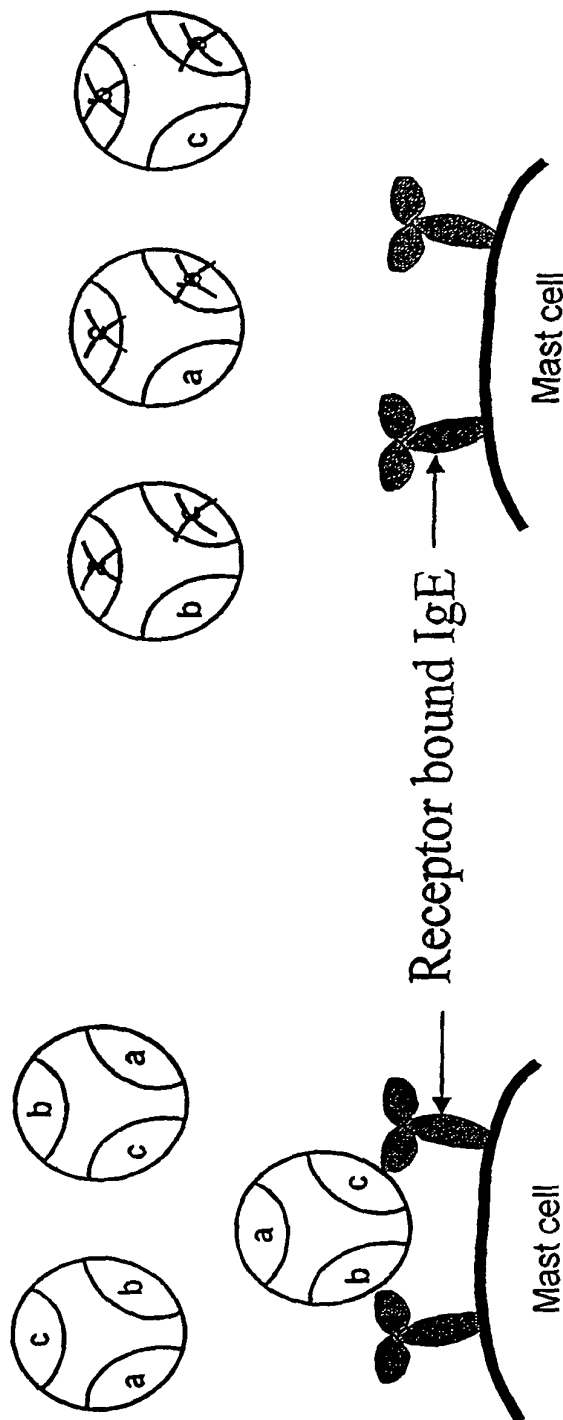


Fig. 14  $\blacklozenge$  r Ves v 5  $\blacksquare$  Lys72Ala  $\blacktriangle$  Tyr96Ala

Fig. 15

Effect of point mutations in dominating IgE epitopes  
hypothetical model with 3 epitopes



Cross-linking

Fig. 15A

No cross-linking

Fig. 15B

Fig. 16

## DNA SEQUENCE

Der p 2 (DNA sequence referred to in notes in accession No. P49278 SWISSPROT)

## ORIGIN

```

1   cacaaattct tctttcttc ttactactga tcattaatct gaaaacaaaa ccaaacaac
61  cattcaaaat gatgtacaaa atttgtgtc tticattgtt ggtcgcagcc gttgctcgtg
121 atcaagtcga tgcacaagat tgtgccaatc atgaaatcaa aaaagtttg gtaccaggat
181 gccatgggtc agaaccatgt atcaticatc gtggtaaacc attccaatg gaagccgttt
241 tcgaagccaa ccaaaacaca aaaacggcta aaattgaaat caaagcctca atcgatgggt
301 tagaagtiga tgttcccggt atcgatccaa atgcatgcca ttacatgaaa tgccattgg
361 ttaaaggaca acaatatgat attaaatata catggaatgt tccgaaaatt gcaccaaaat
421 ctgaaaatgt tgcgtcact gftaaagtta tgggtgatga tgggtgttg gcctgtgcta
481 ttgtactca tgctaaaatc cgcgatteaa tcaaacaaaa ttattgatt ttgtaatcac
541 aaatgattga ttttcttcc aaaaaaaaaa taaataaaat ttgggaatt c

```

## AMINO ACID SEQUENCE

Der p 2 (Accession No. P49278 SWISSPROT; includes signal peptide 1-17)

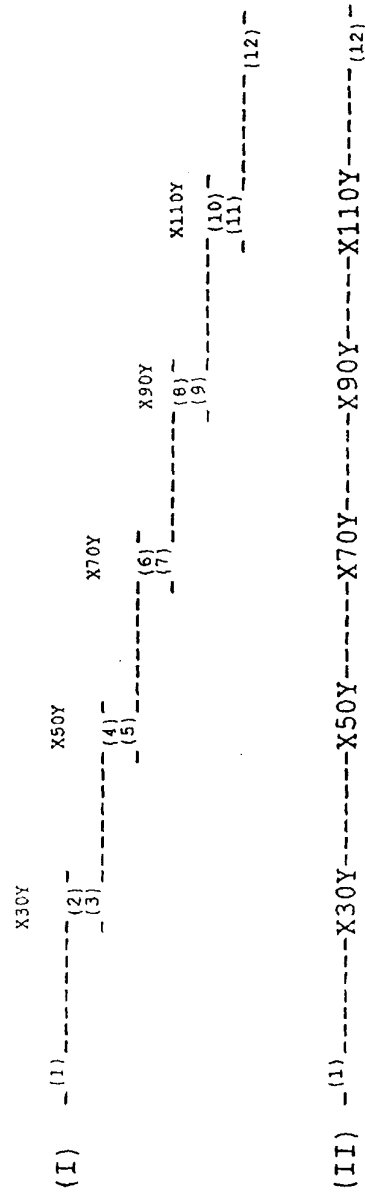
```

1   mmykilclsl lvaavardqv dvkdcanhei kkvlvpgchg sepcihrhk pfqleavfea
61  nqntktakie ikasidglev dvpgidpnac hymkcplvkg qqydikytwn vpkiaapksen
121 vvvtkvmgd dgvlacaiat hakird

```



Figure 17



Lines represents DNA sequences.

Numbers in parentheses above lines represents sense oligonucleotide primers: (1), (3), (5), (7), (9), (11).  
 Numbers in parentheses below lines represents anti-sense oligonucleotide primers: (2), (4), (6), (8), (10), (12).  
 Notation X (position) Y represents mutations.

(1) Represents the sense oligonucleotide primer accommodating the protein N-terminus.

(12) Represents the anti-sense oligonucleotide primer accommodating the protein C-terminus.

# Genetic Characterization

Figure 18

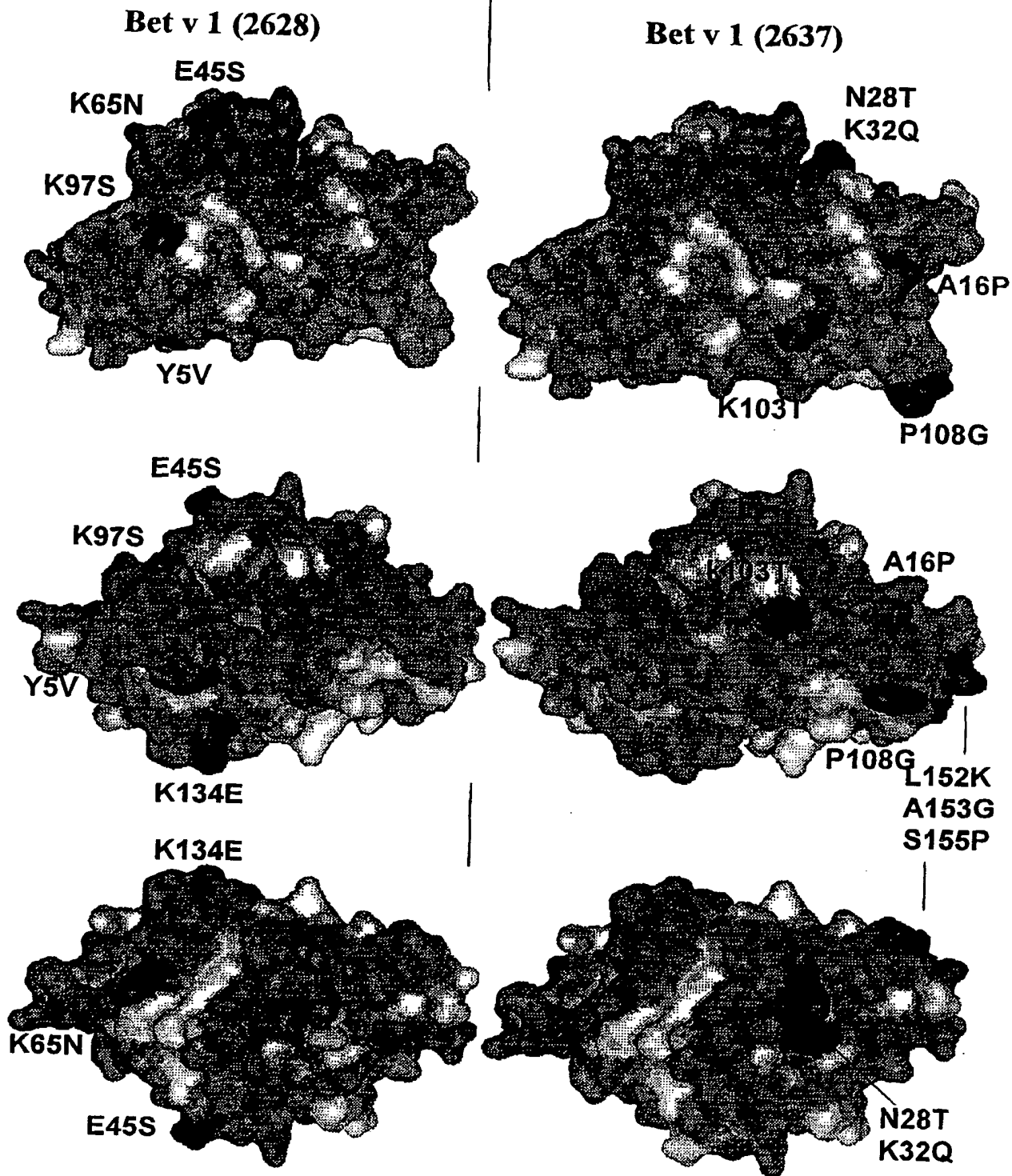
Bet v 1 (2637)

(A16P, N28T, K32Q, K103T, P108G, L152K, A153G, S155P)

DNA template: Bet v 1 (2571) carrying N28T, K32Q, P108G mutations.

331pMalc (s) 189BV (a) 368BVa 331pMalc : CAGACTAATTCGACGTCGGTACCC  
 188BV (s) 362BV (a) 367BVa 368BVa : CAGTCGcggtGCTGGGATAACAGA  
 361BV (s) 364BV (a) 369BVa 367BVa : CCAGCACcgCGACTGTTCAAGGCC  
 363BV (s) 365BV (a) 370BVa 370BVa : CACTATggtTATCTCGTTGGAGAT  
 366BV (a) 366BV (a) 372BVa 369BVa : GAGATAaccATAGTGGCAActggt  
 365BV (s) 332pMalc (a) 372BVa: TTACTGAATTCATTAGTTGTAGGCATCcggtGgcctttGAGGTA

331pMal c : CAGACTAATTCGAGCTCGGTACCC  
 189BV : TTTCCTGAAATGTTTCAACACT  
 188BV : AACATTCAGGAAATGGAGGGCC  
 362Bva : CACGTAGTTGAAAGGGAGGCCTTC  
 361BVa : TTTCAACTACGTGAAGGACAGAGT  
 364Bva : GGAGATGCTCTCCAATGTGTGCGCC  
 363BVa : GGAGAGCATCTCCAACGAGATAAA  
 366Bva : ACTTGCTTCAACCTGCTCTGCCTT  
 365BVa : CAGGTTGAAGCAAGTAAAGAAATG  
 332pMal c : GCAGGTCGACTCTAGAGGATCCAT



Molecular surface of Bet v 1.

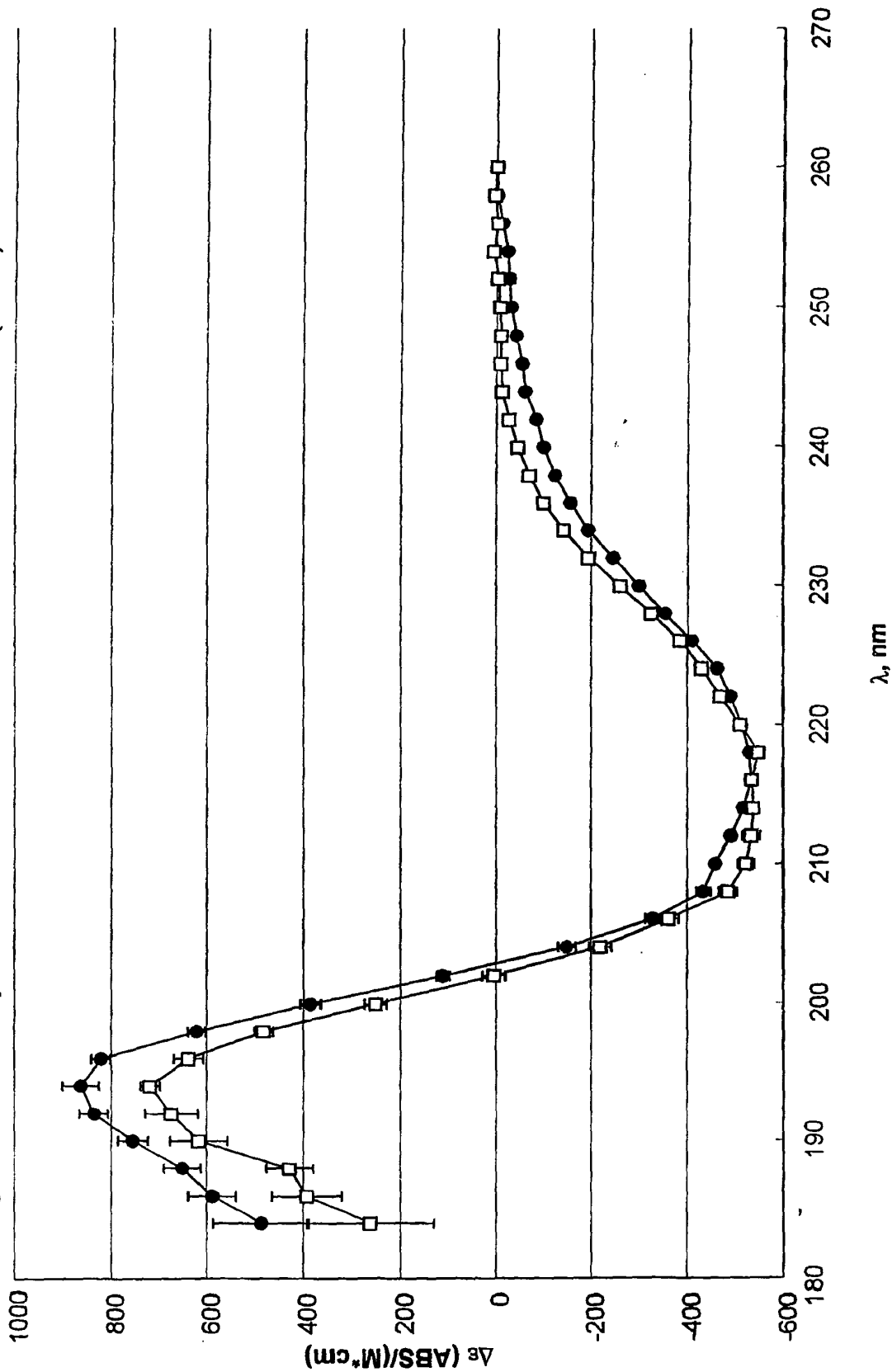
Left side: Bet v 1 (2628), Right side: Bet v 1 (2637)

Grey: Backbone + amino acids 95-100% conserved among *Fagales*

Black: Introduced point mutations.

Figure 19

Figure 20 CD-spectrum of Bet v 1.2801, and mutant Bet v 1 (2637)



CD-spectrum of Bet v 1 (2637), open squares, and the CD-spectrum of native folded Bet v 1.2801, closed circles, both obtained at 20 °C

**Inhibition of human serum IgE-binding to Bet v 1.2801  
with Bet v 1.2801 and mutated Bet v 1 allergens**

Figure 21

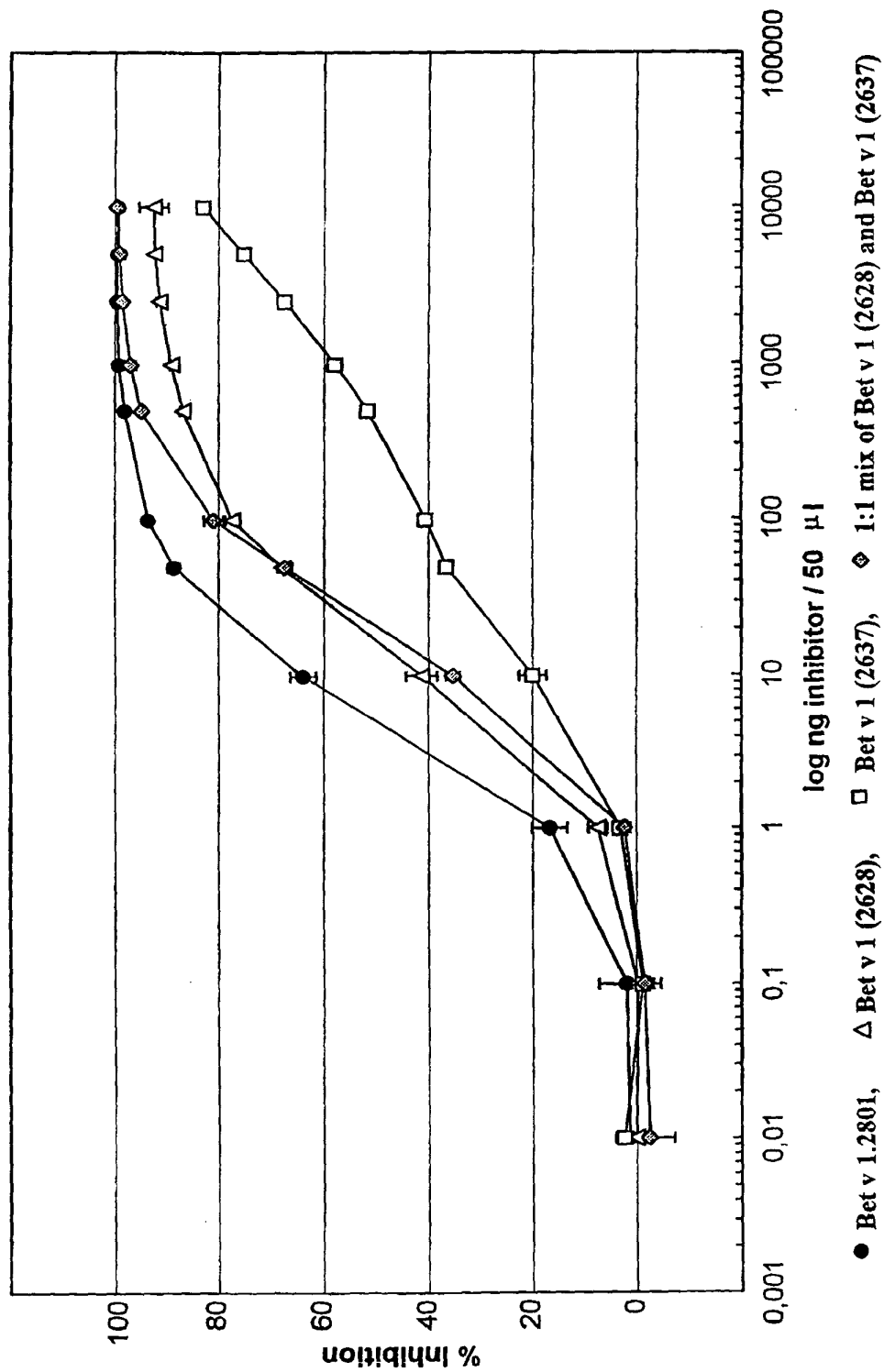


Figure 22 Histamine release, donor MCDS, Bet v 1.2801, Bet v 1(2628), Bet v 1 (2637)

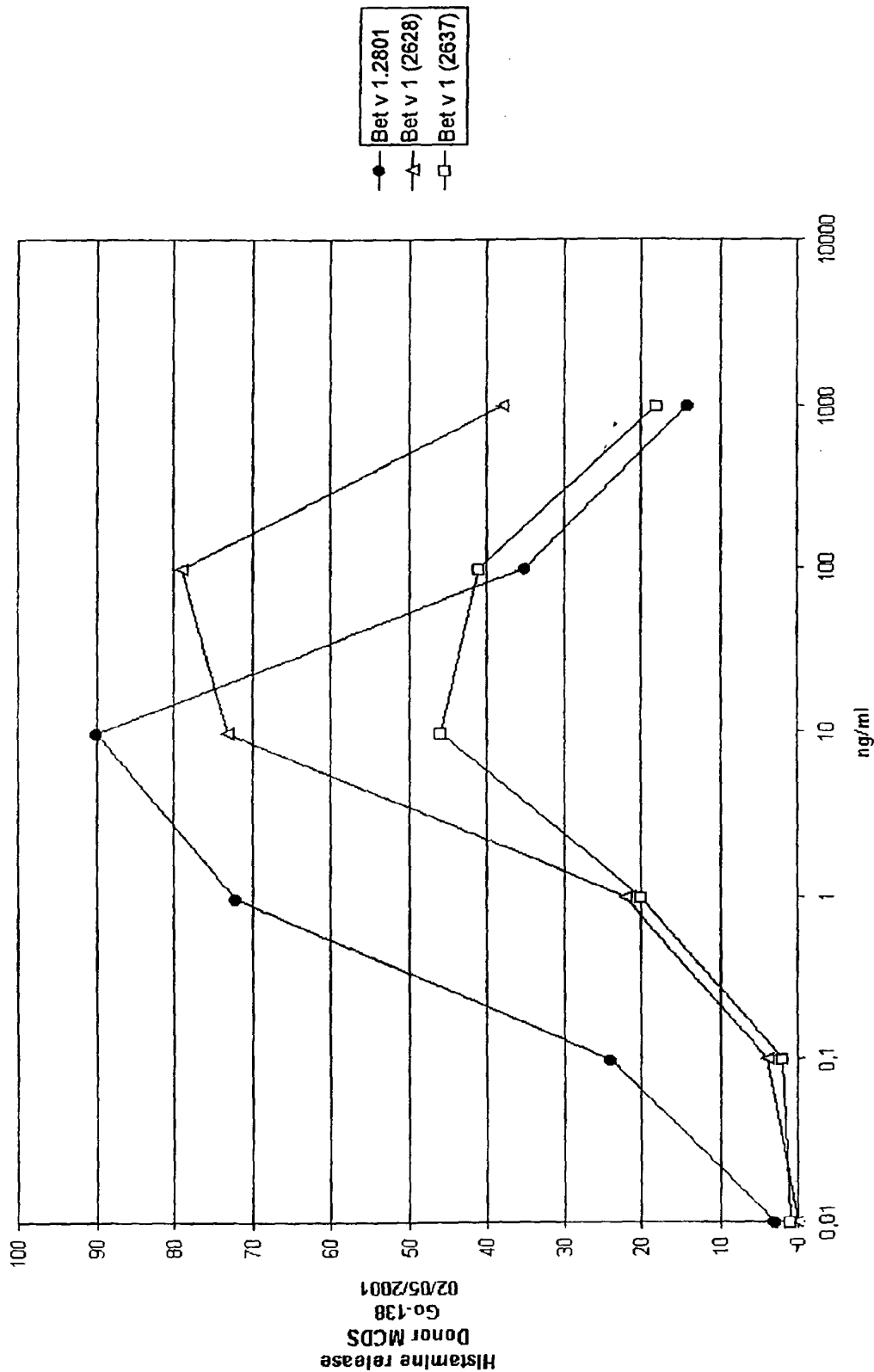


Figure 23 Histamine release, donor MDH, Bet v 1.2801, Bet v 1(2628), Bet v 1 (2637)

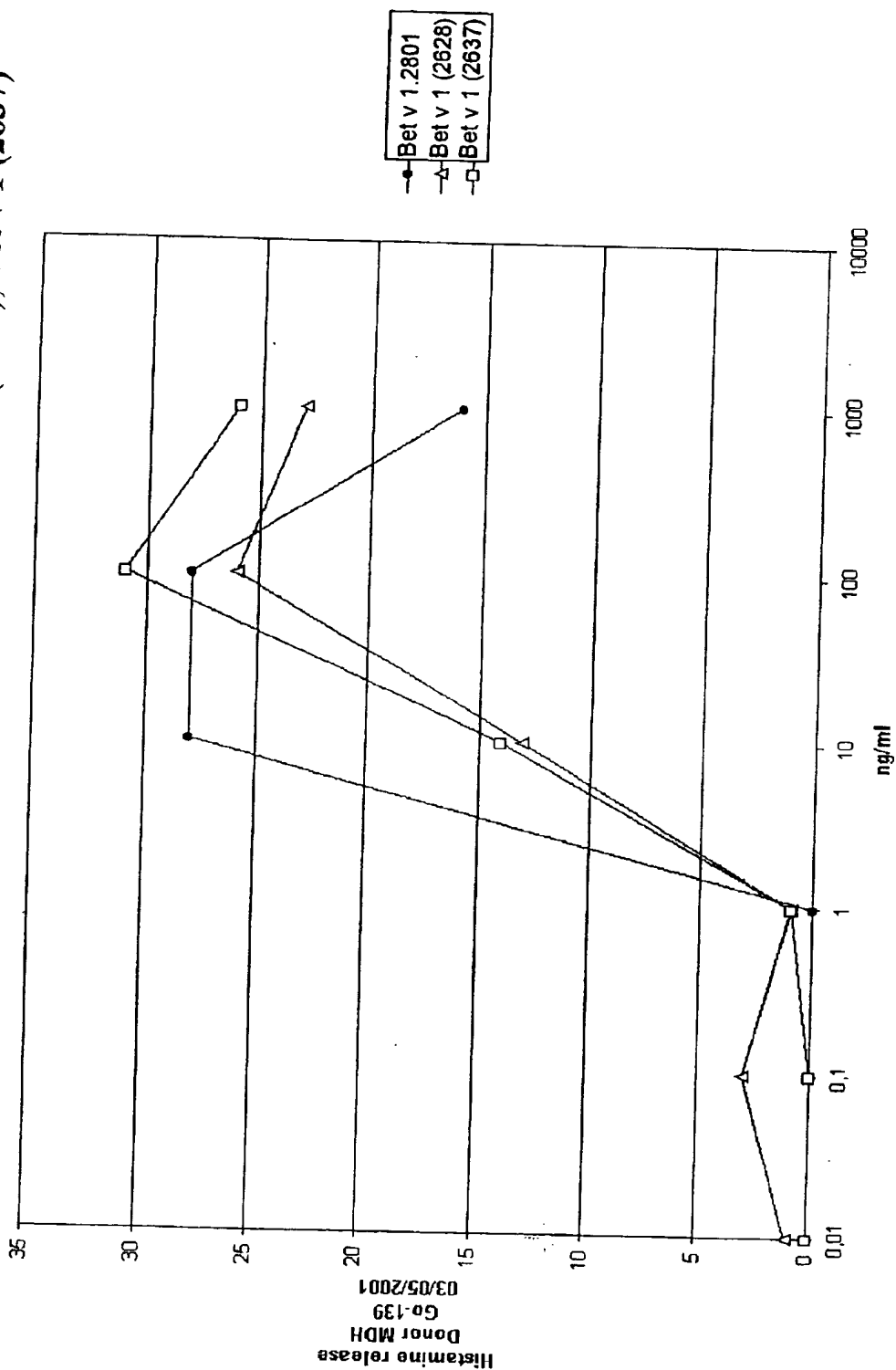
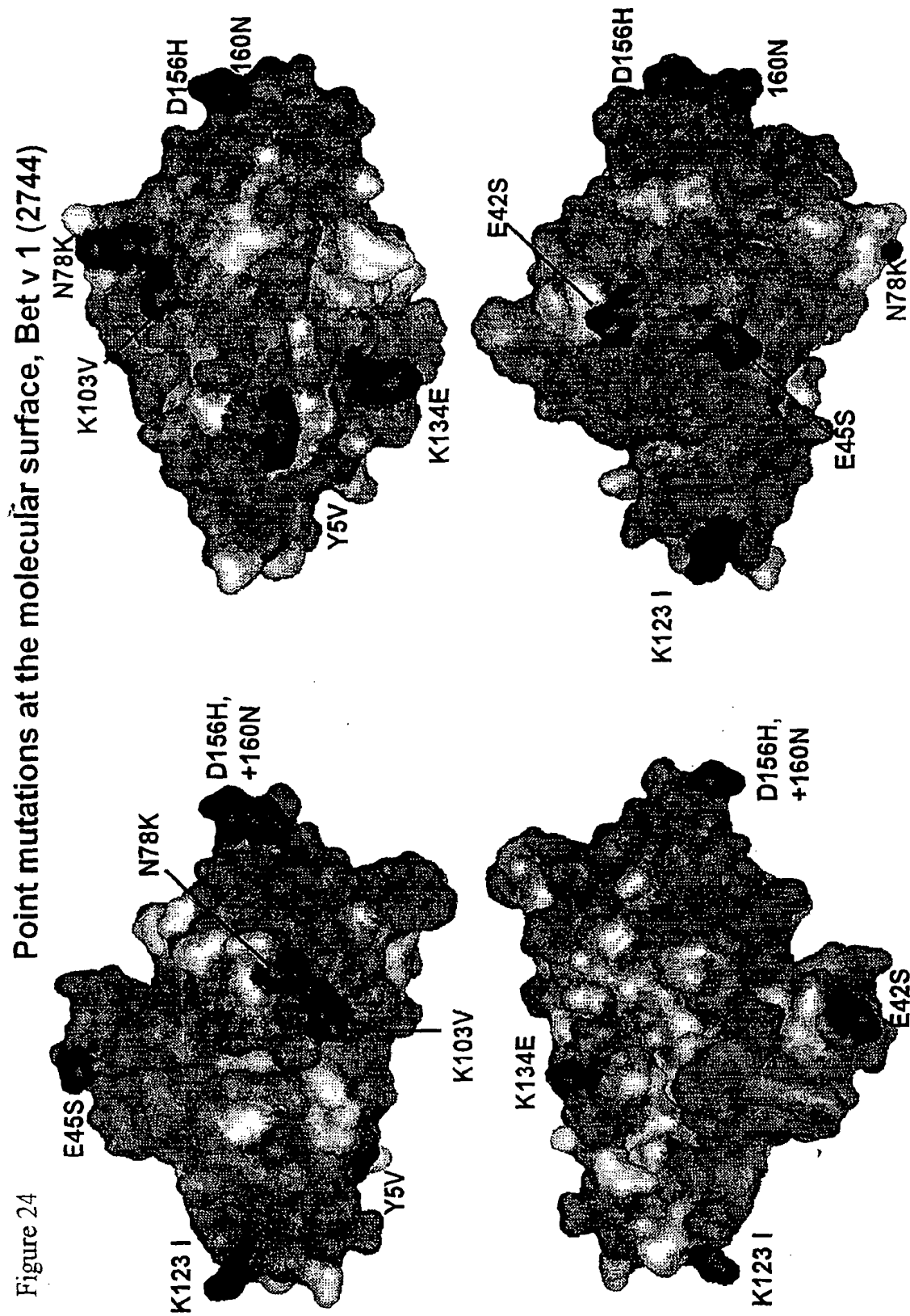
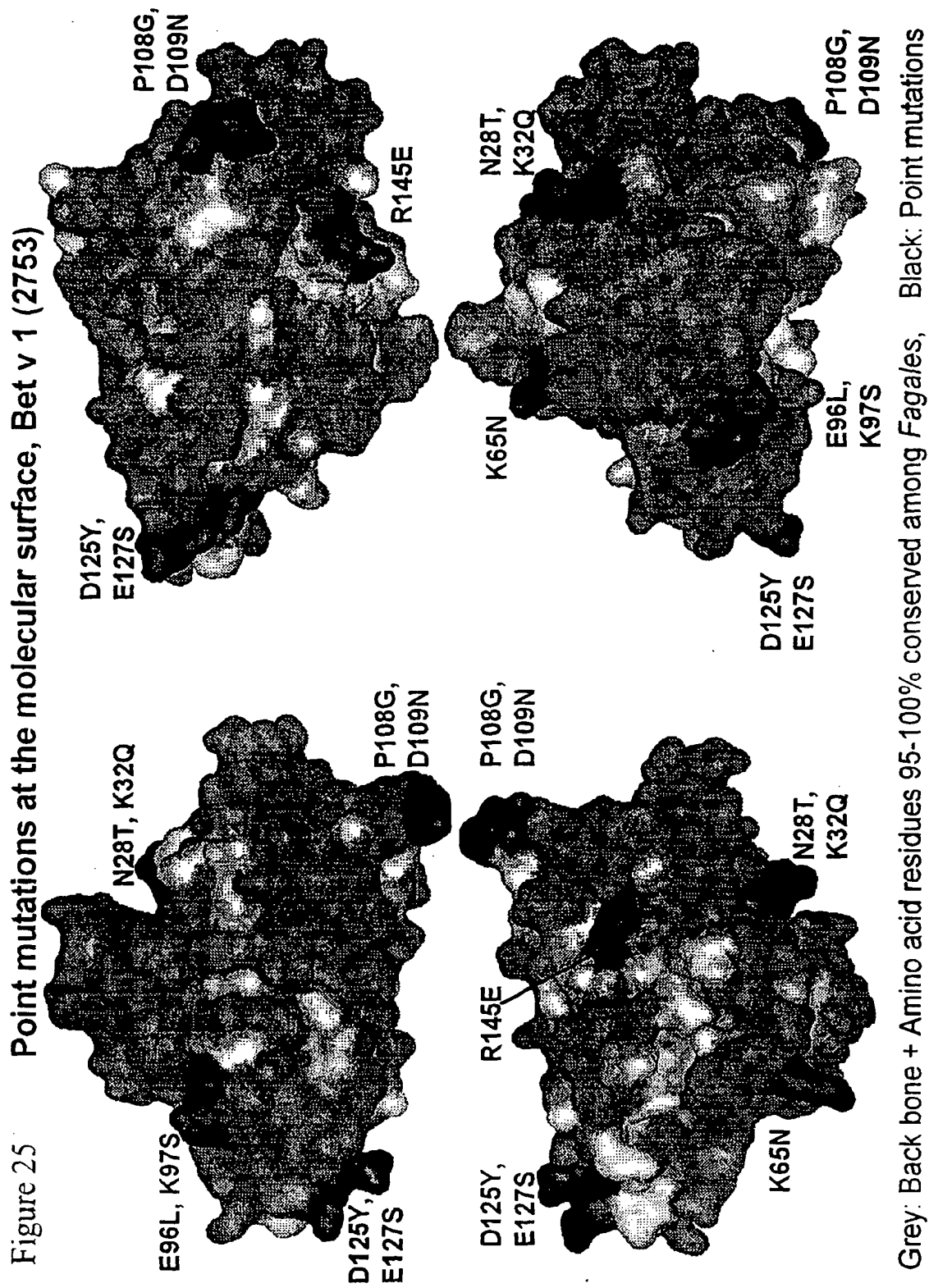


Figure 24

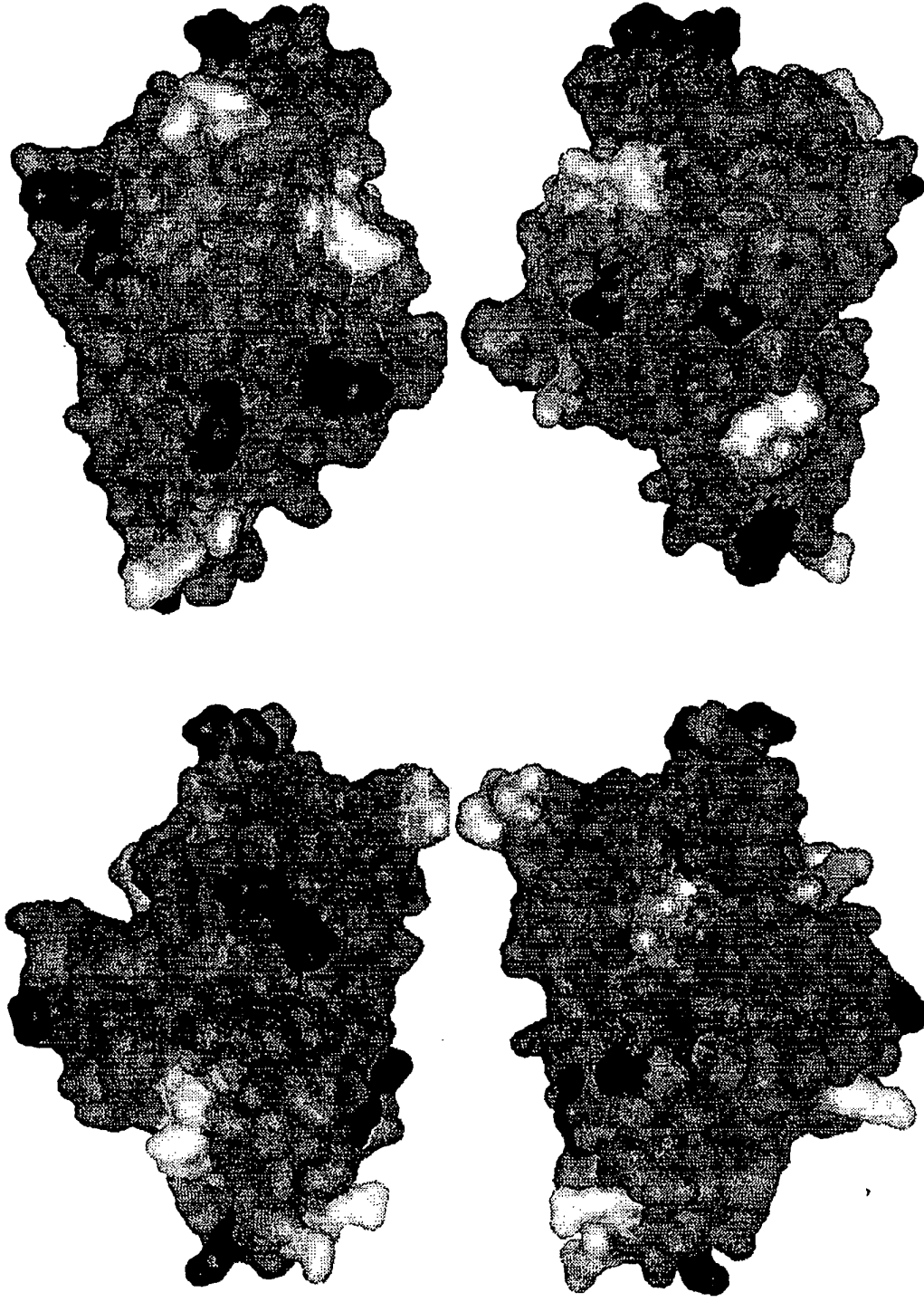


Grey: Back bone + Amino acid residues 95-100% conserved among *Fagales*, Black: Point mutations





Distribution of point mutations at the molecular surface of, Bet v 1 (2744) [white], and Bet v 1 (2753) [Black]



Grey: Molecular surface; amino acid residues 95-100% conserved among *Fagales*  
 Black: Mutations (Y5V, K134E), (E42S, E45S), (N78K, K103V), K123 I, (D156H, +160N)  
 White: Mutations (N28T, K32Q), K65N, (E96L, K97S), (P108G, D109N), (D125Y, E127S), R145E

Figure 26

Figure 27 Circular dichroism spectra of Bet v 1.2801 and mutant Bet v 1(2744), pH 7.13, T 20C.

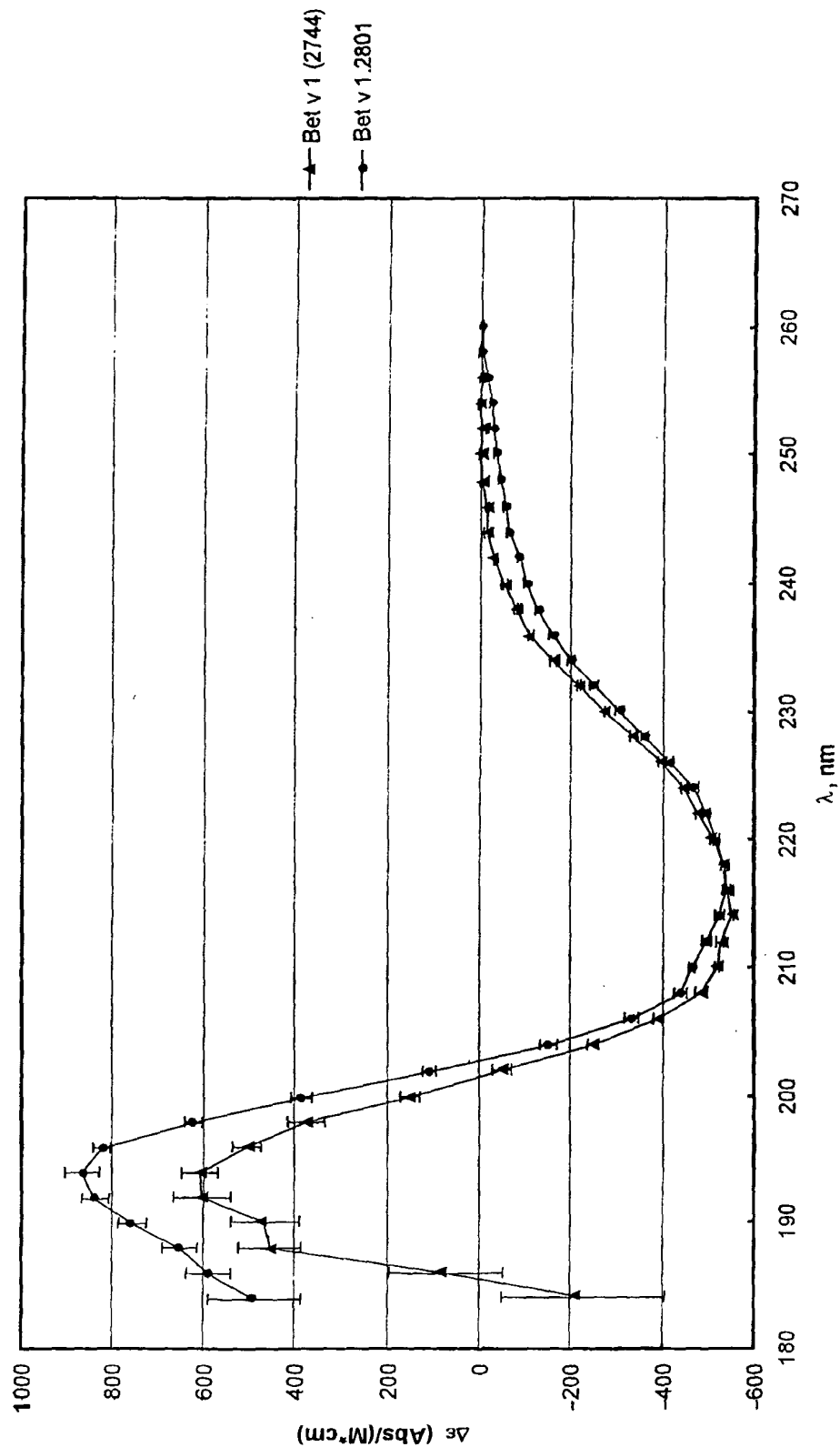


Fig. 28: Histamine release, donor MK, Bet v 1.2801 and Bet v 1(2744)

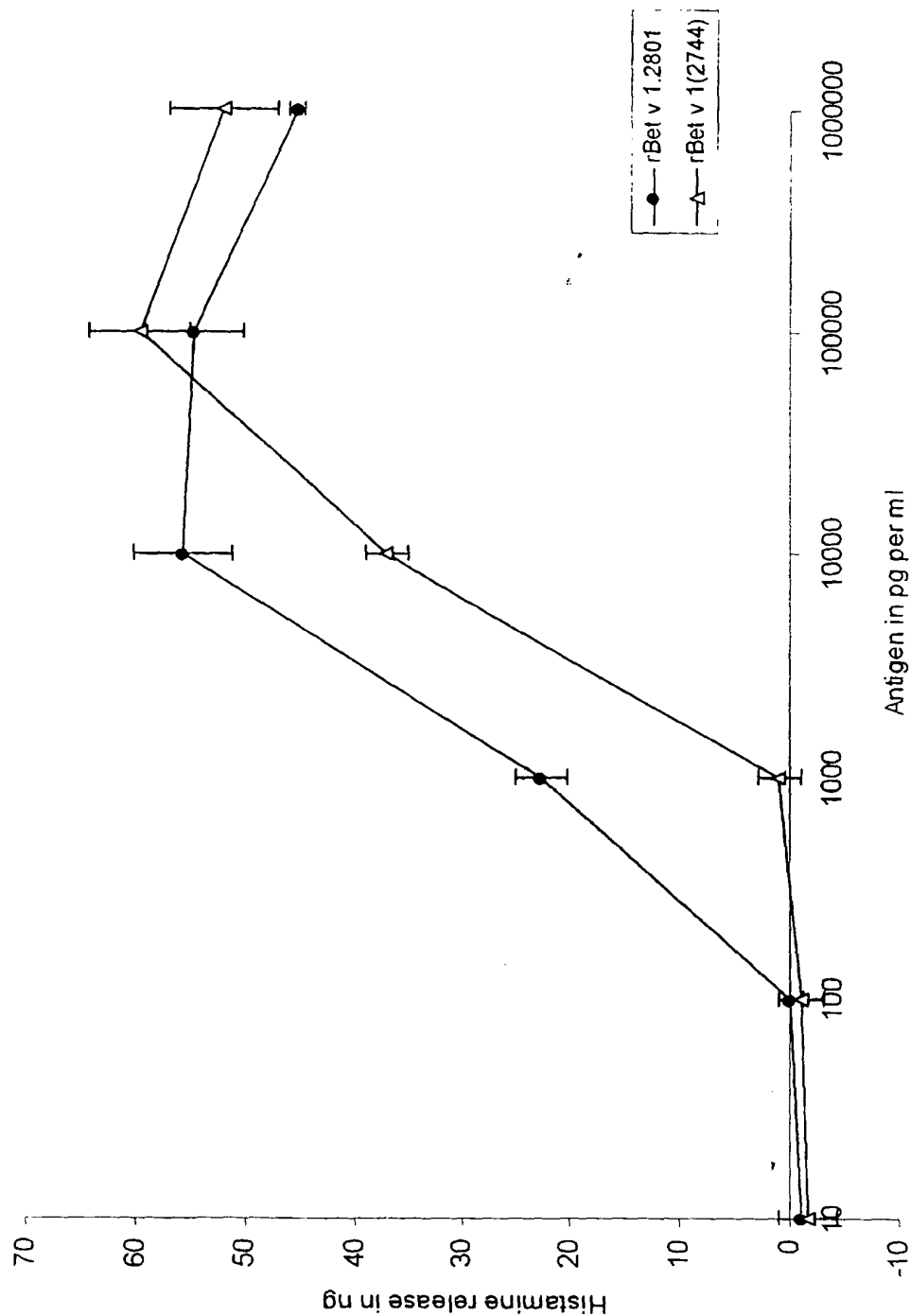


Fig. 29A: Histamine release, donor MJ, Bet v 1.2801 and Bet v 1 (2744)

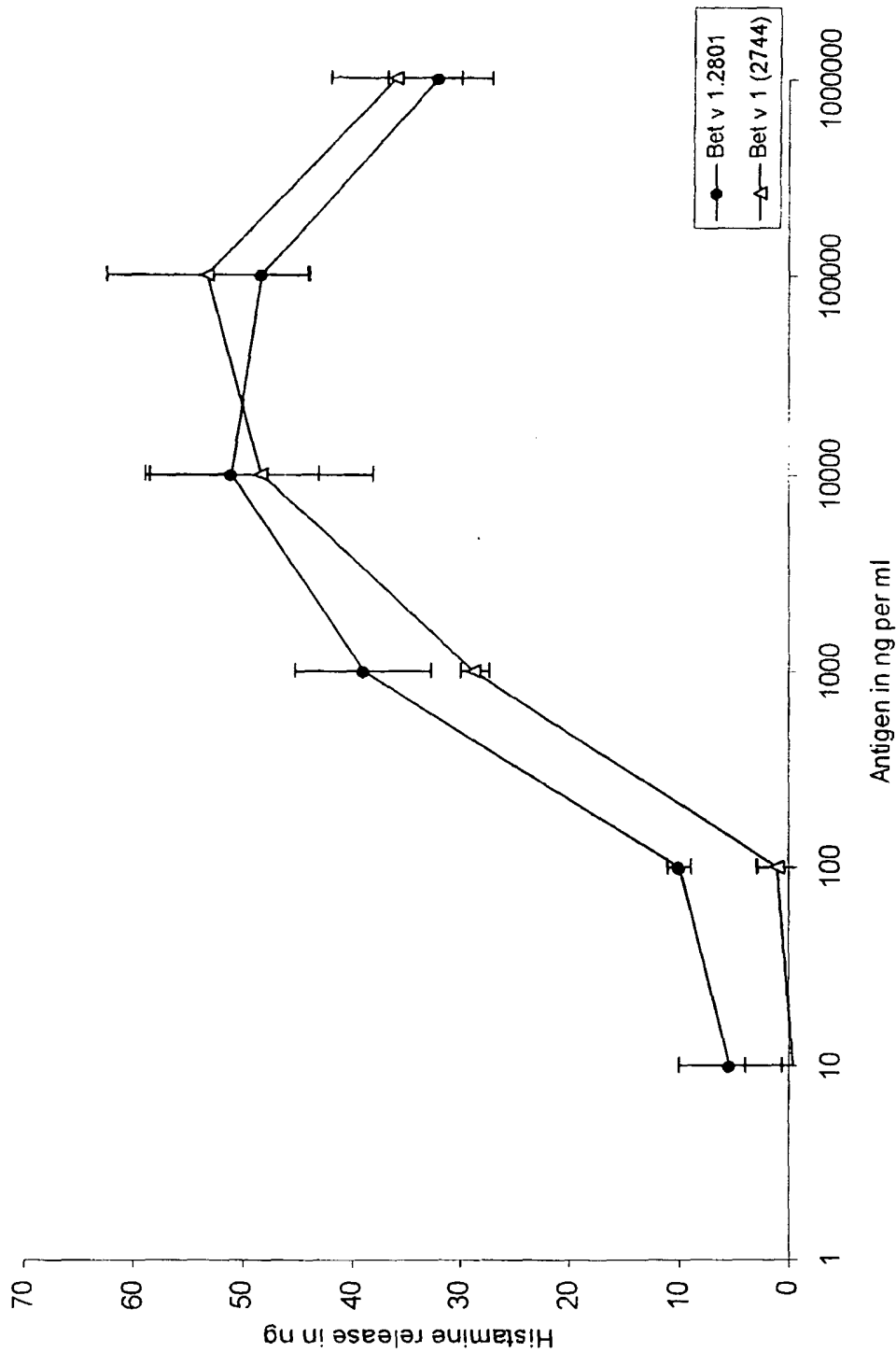


Fig.29B: Histamine release, donor MH, Bet v 1.2801 and Bet v 1 (2744)

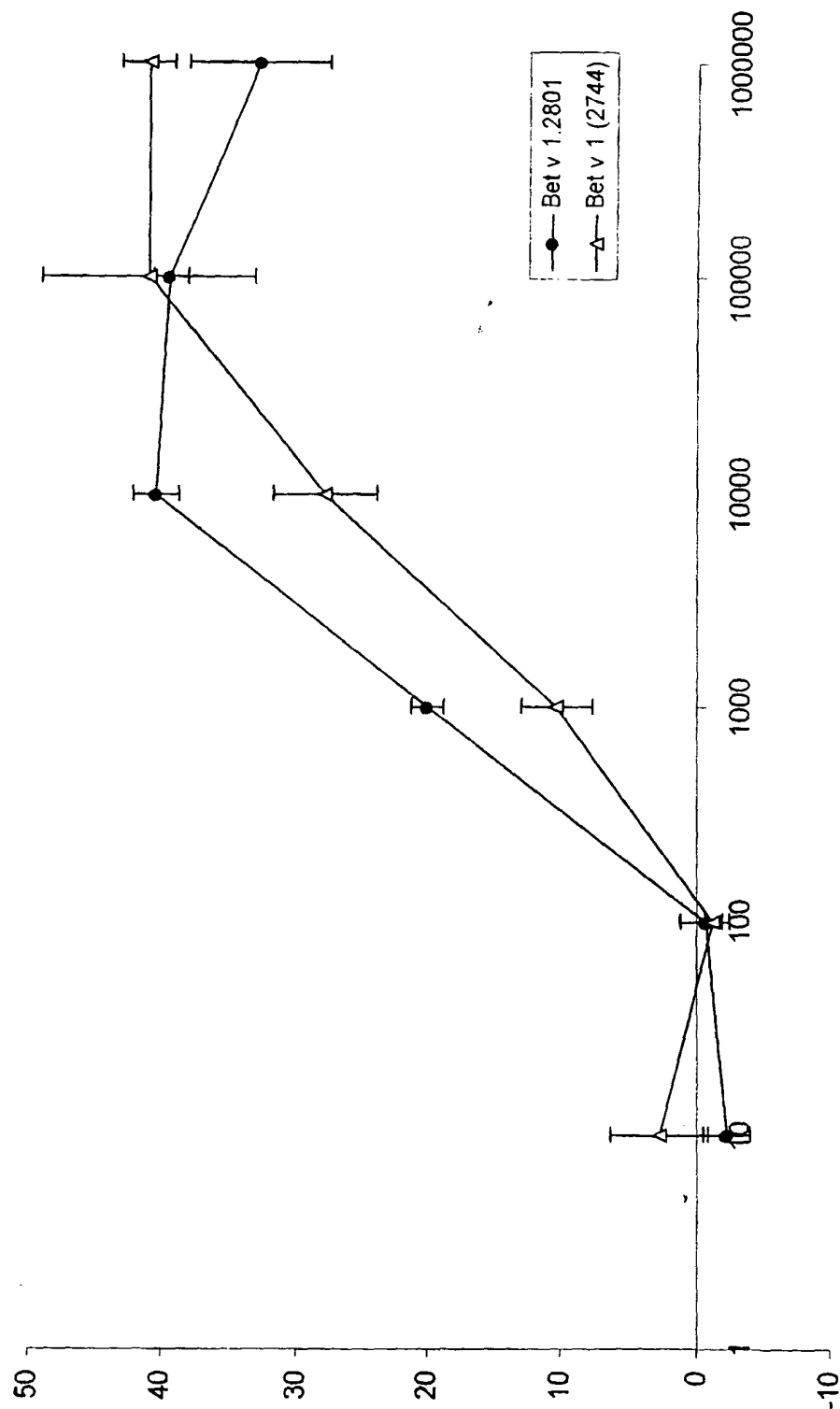


Fig. 29C: Histamine release, donor CJB, Bet v 1.2801 and Bet v 1 (2744)

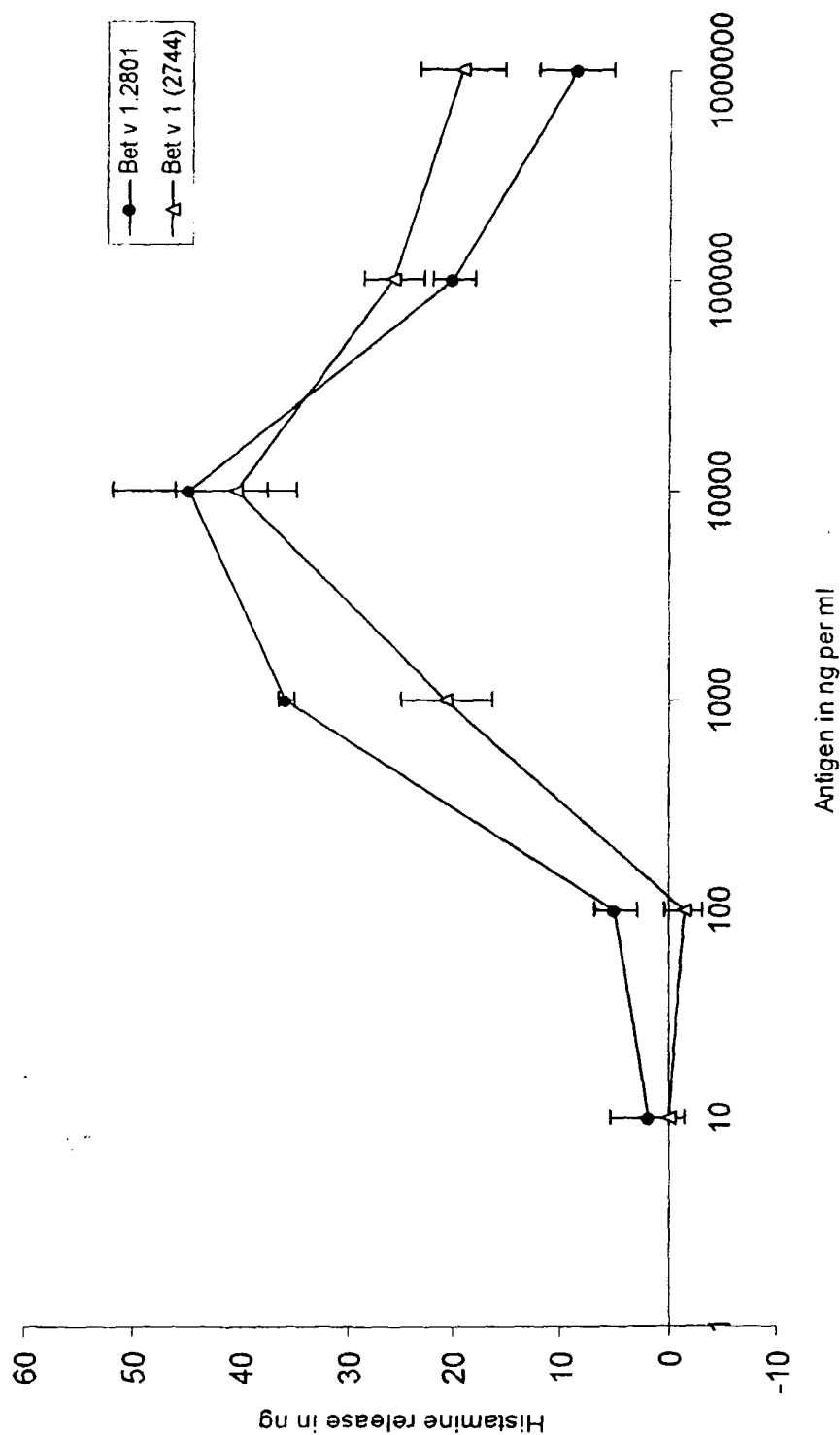
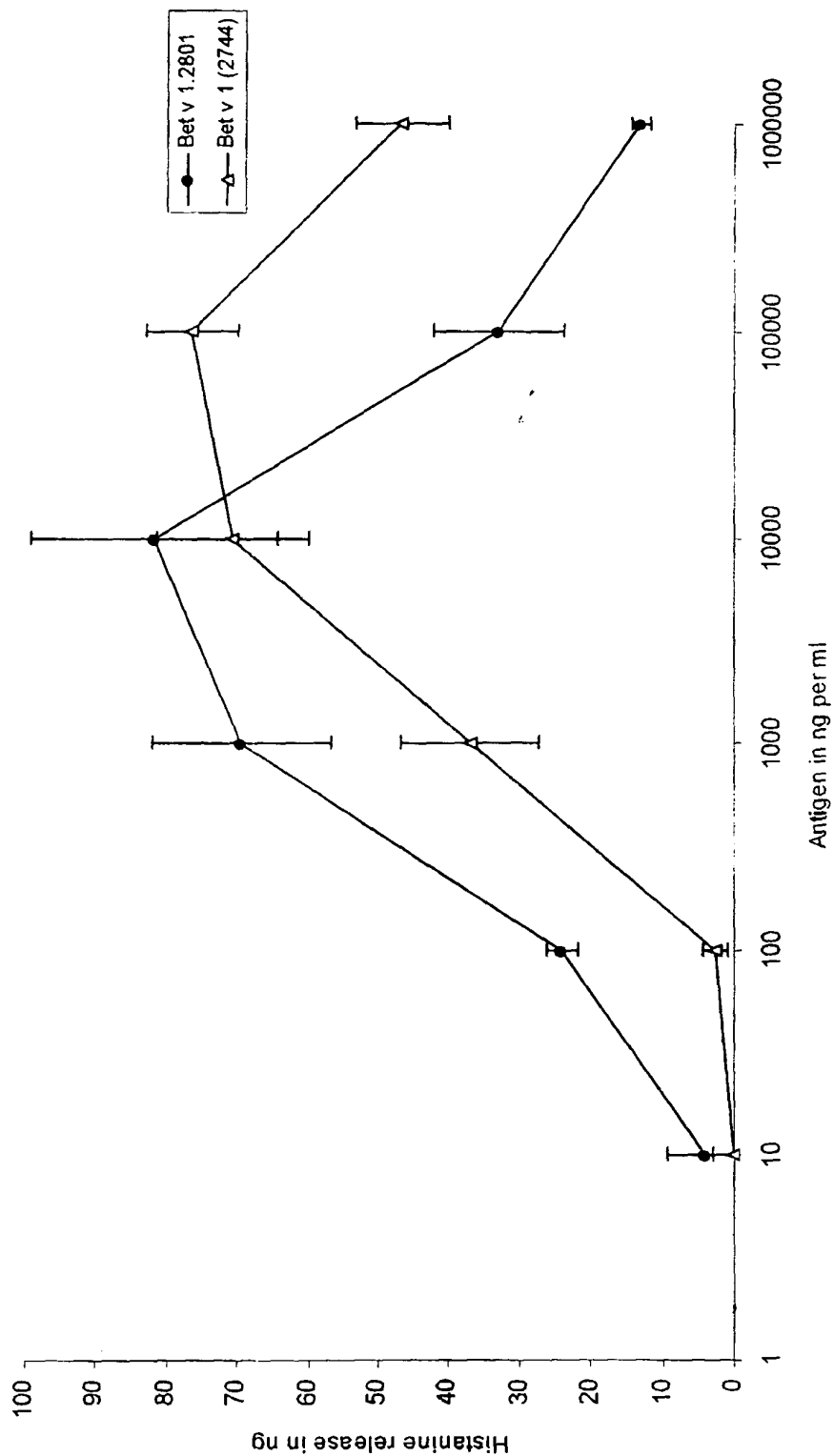


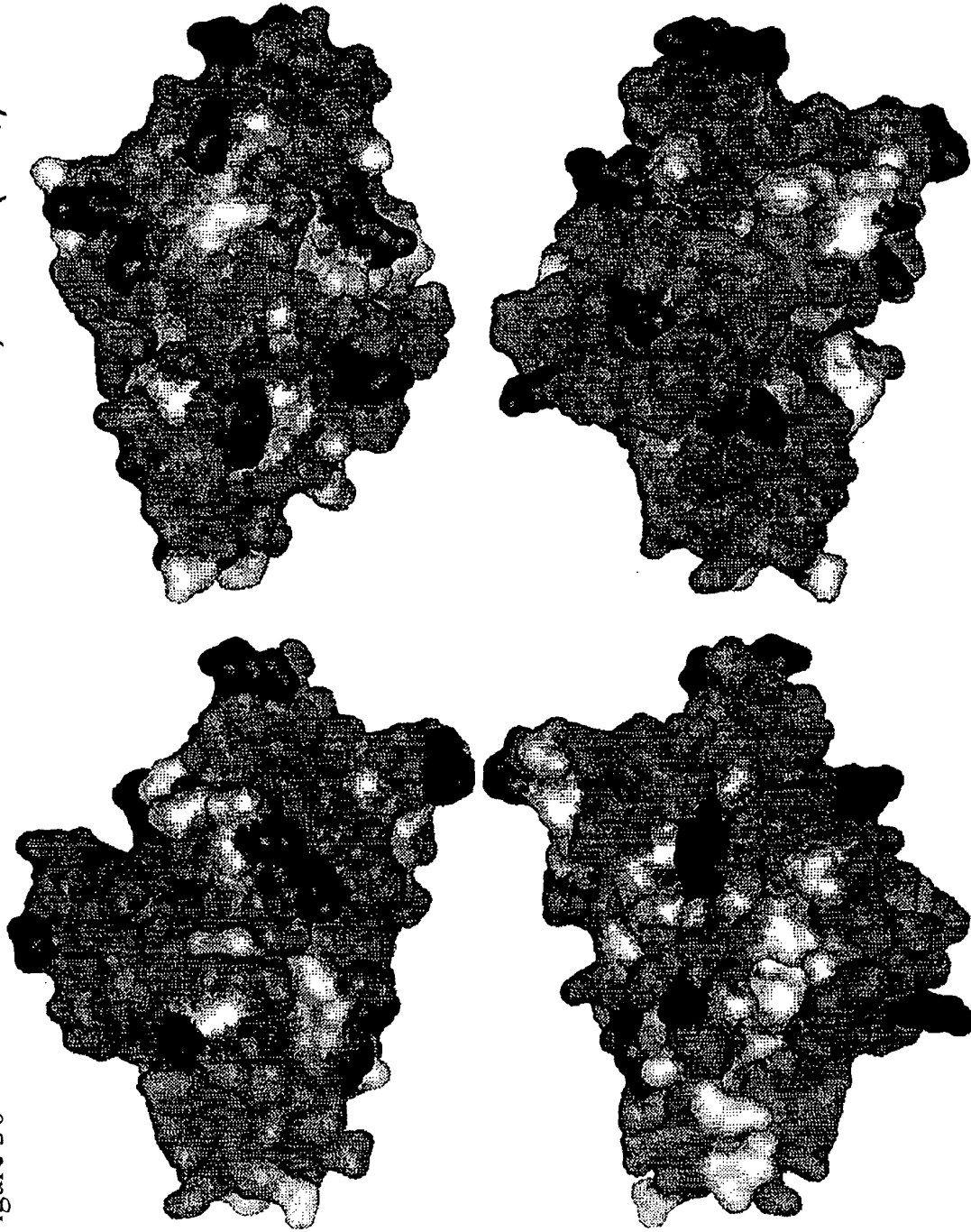
Fig. 29D: Histamine release, donor MCDS, Bet v 1.2801 and Bet v 1 (2744)





FOOT SHEET

Figure 30 Point mutations at the Molecular surface, Bet v 1 (2733)



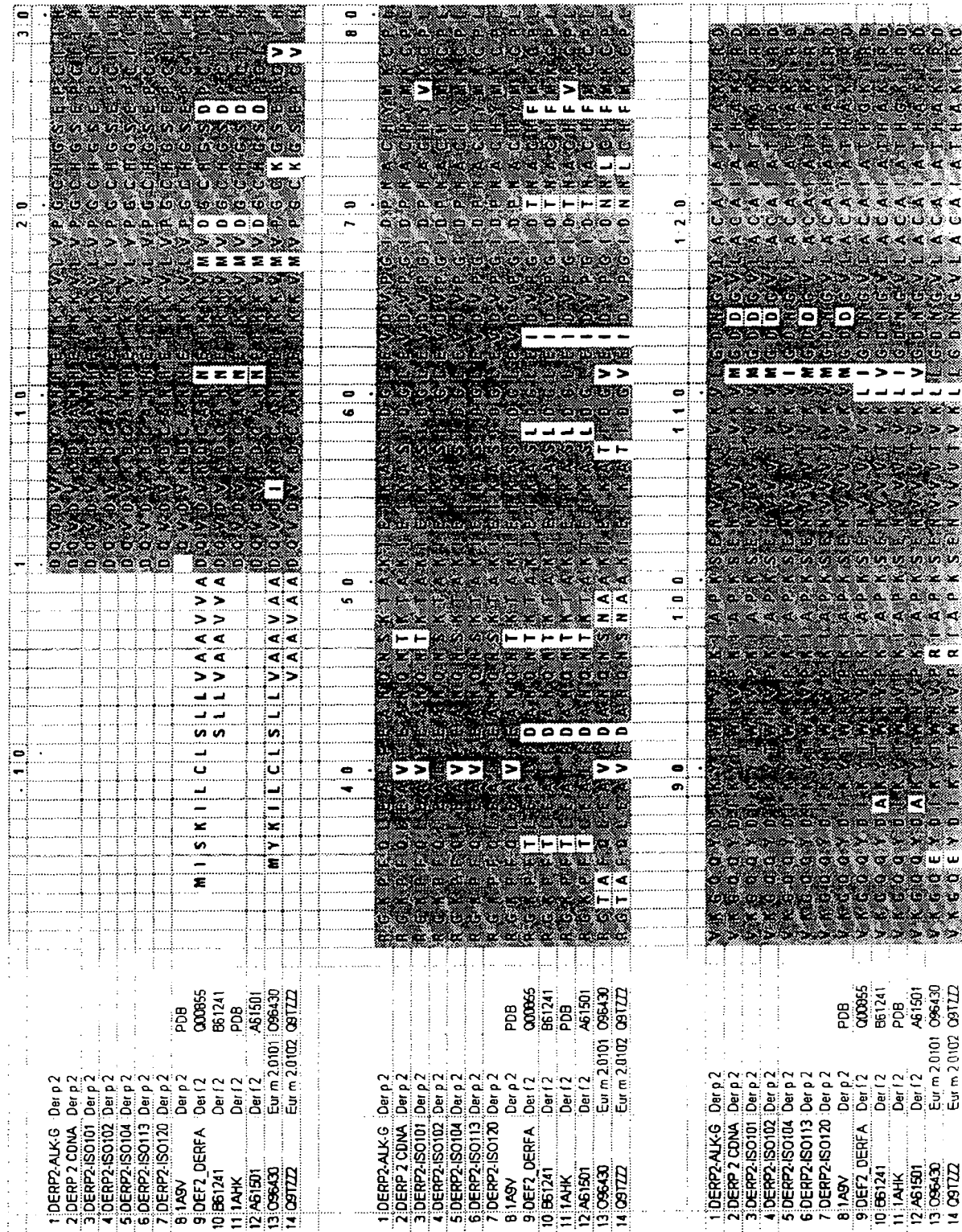
Grey: Back bone + Amino acid residues 95-100% conserved among *Fagales*,  
Black: Point mutations: Y5V, N28T, K32Q, E45S, K65N, N78K, K97S, P108G, K134E, R145E, D156H, +160N

**Figure 31**

Oligonucleotide primers for site-directed mutagenesis of Der p 2

K6A	sense	OB43	42-mer	5' -CCGCTCGAGAAAAGAGATCAAGTCGATGTCGCCGATTGTGCC- 3'
	anti-sense	OB28	39-mer	5' -CGTTCCTAGACTATTAATCGCGGATTTTAGCATGAGTTGC- 3'
K15E	sense	OB44	67-mer	5' -CCGCTCGAGAAAAGAGATCAAGTCGATGTCAAAGATTGTGCC AACCATGAAATCAAAGAAGTTTGG- 3'
	anti-sense	OB28	39-mer	5' -CGTTCCTAGACTATTAATCGCGGATTTTAGCATGAGTTGC- 3'
H30N	sense	OB46	54-mer	5' -CGGGGTACCAGGATGTCATGGTTCAGAACCATGTATCATTAA CCGTGGTAAACC- 3'
	anti-sense	OB28	39-mer	5' -CGTTCCTAGACTATTAATCGCGGATTTTAGCATGAGTTGC- 3'
E62S	sense	OB47	33-mer	5' -GCCTCAATCGATGGTTTATCAGTTGATGTTCCC- 3'
	anti-sense	OB48	33-mer	5' -GGGAACATCAACTGATAAACCATCGATTGAGGC- 3'
H74N	sense	OB49	32-mer	5' -CATGGCATGCAATTACATGAAATGCCCATTTGG- 3'
	anti-sense	OB28	39-mer	5' -CGTTCCTAGACTATTAATCGCGGATTTTAGCATGAGTTGC- 3'
K82N	sense	OB50	50-mer	5' -CTACGCATGCCATTACATGAAATGCCCATTTGGTTAATGGACAA CAATATG- 3'
	anti-sense	OB28	39-mer	5' -CGTTCCTAGACTATTAATCGCGGATTTTAGCATGAGTTGC- 3'

Figure 32 (Der p 2)



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10051245 " 4 4 534

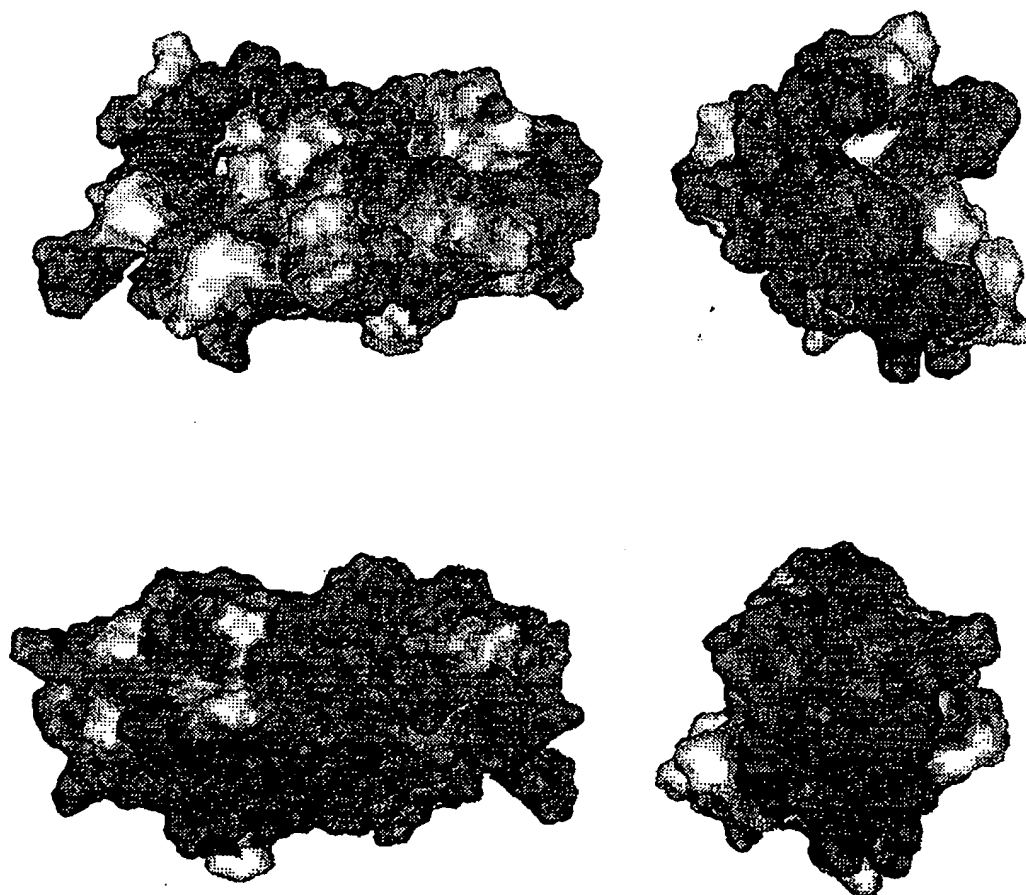


FIG. 33: Der p 2

FIG. 34: Der p 2 mutant

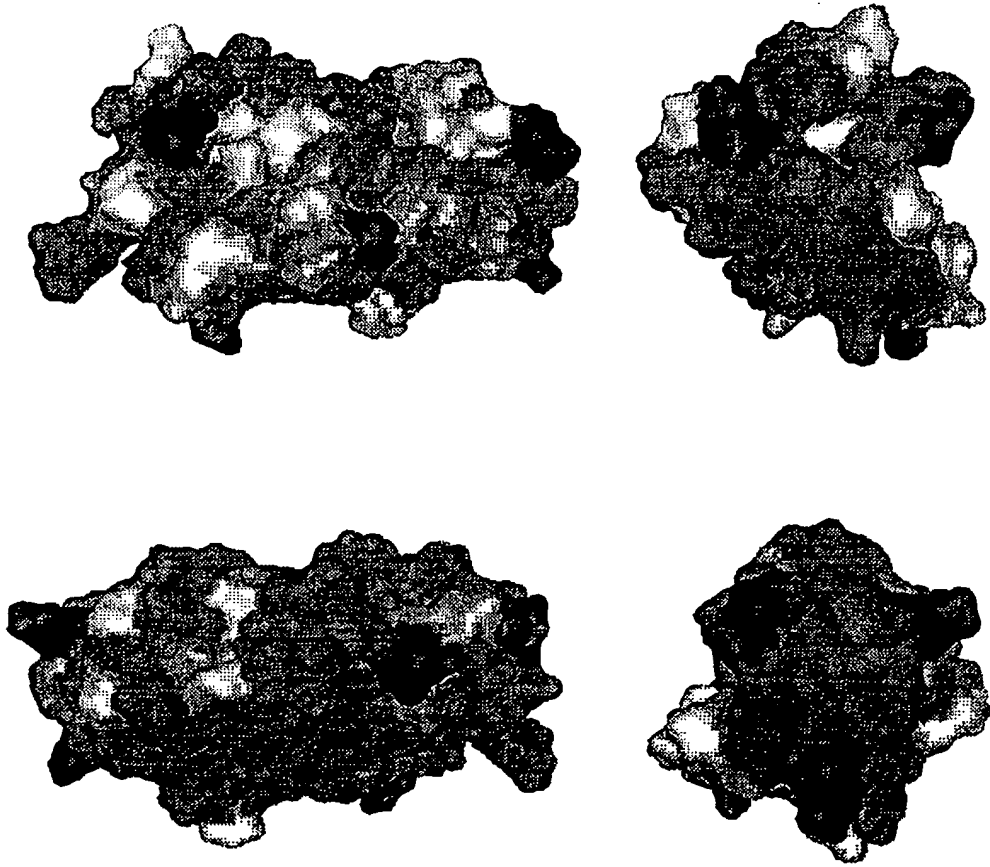
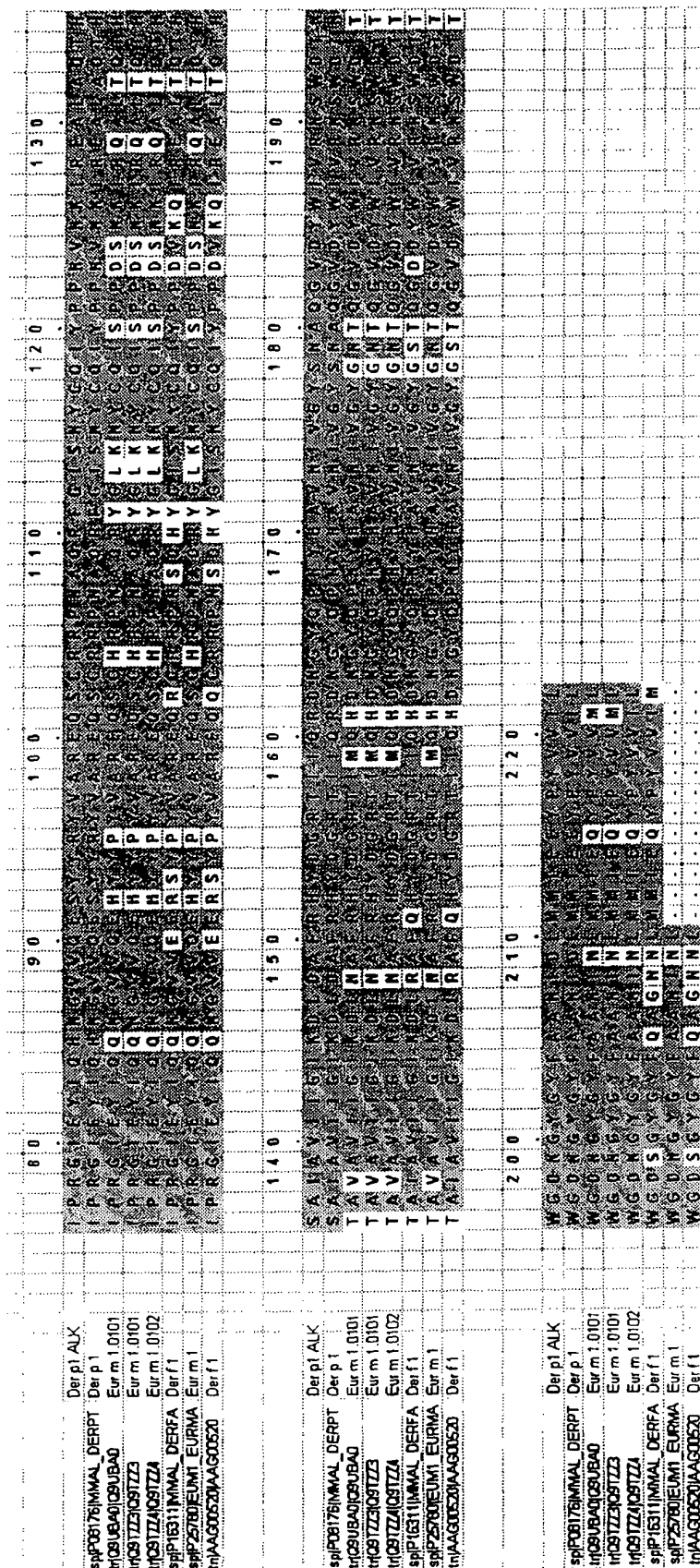


FIG. 34: Der p 2 mutant



FOSTT SH21000

Figure 35B (Der p 1)



10001245 11501  
10511 5421001

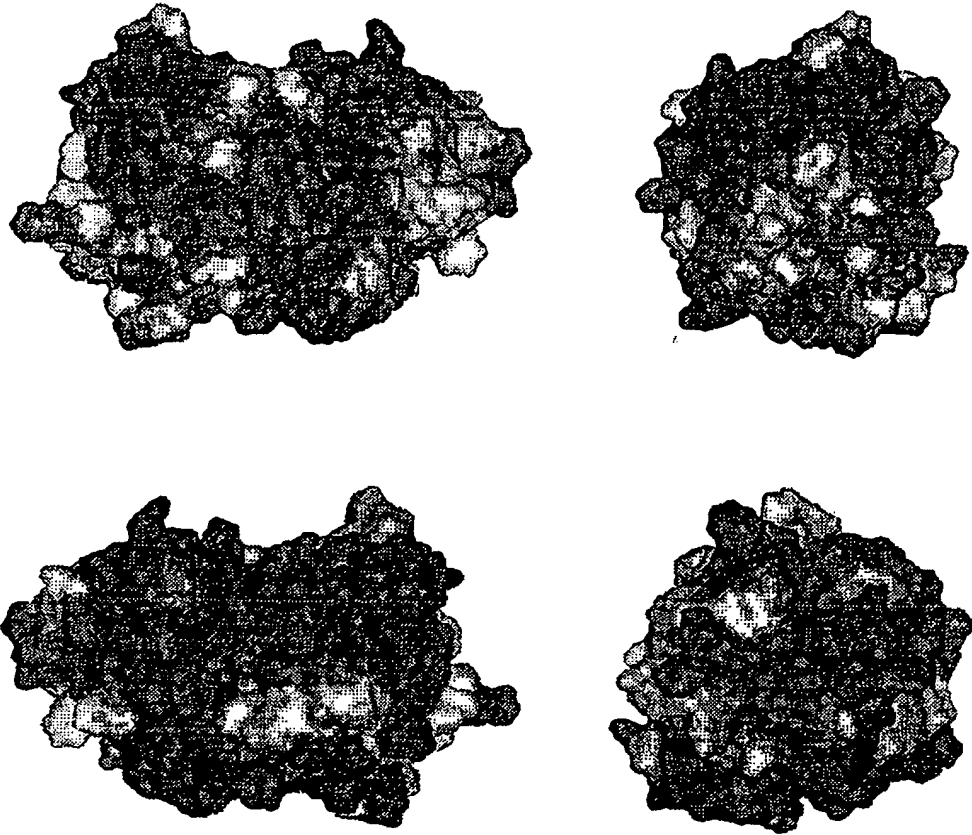


FIG. 36: Der p 1



FIG. 37: Der p 1 mutant

FIG. 38A (Phl p 5)

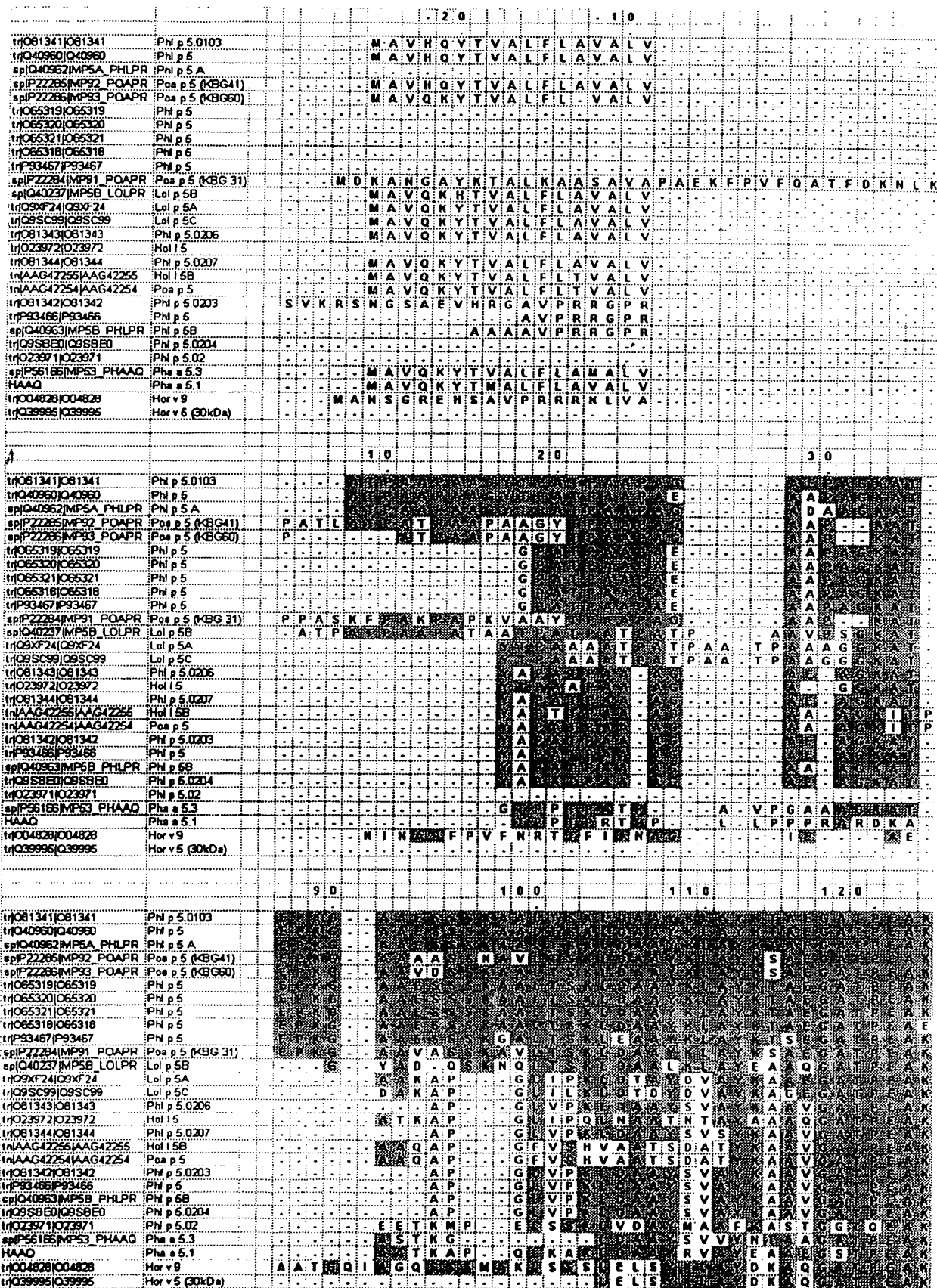
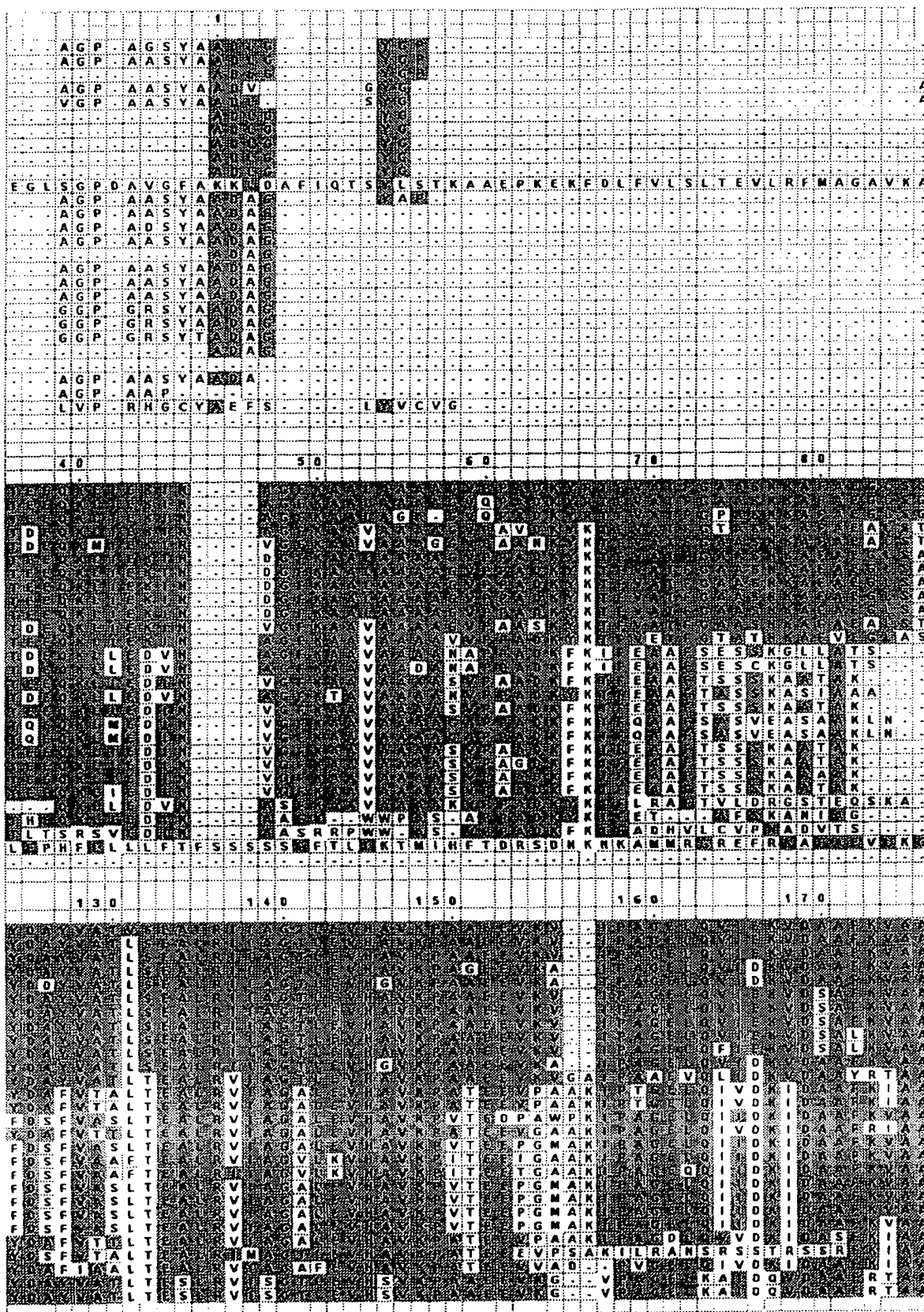


FIG. 38B (Phi p 5)



0001245 11501

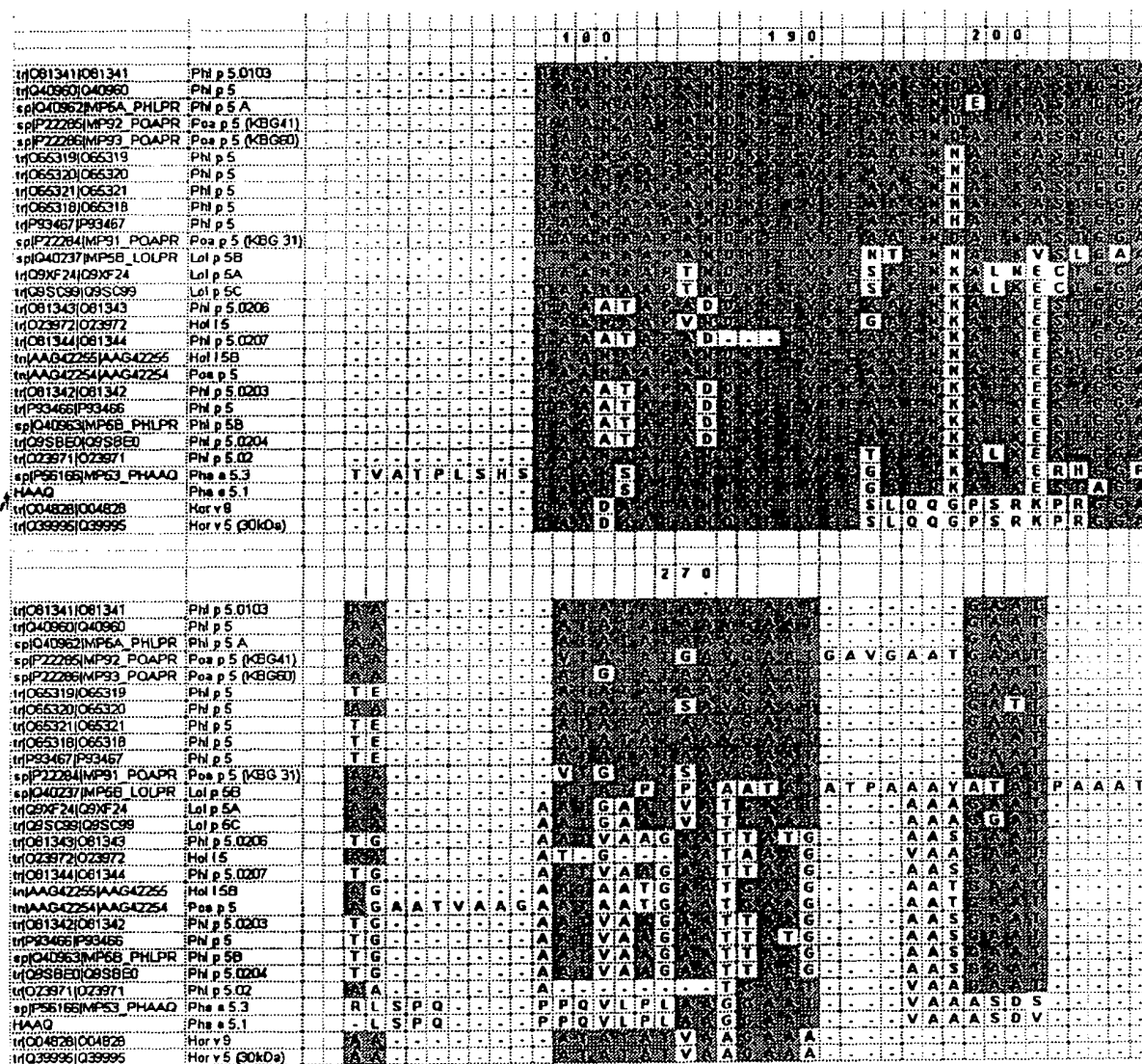
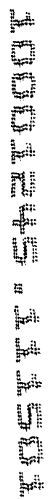


FIG. 38D (Phl p 5)



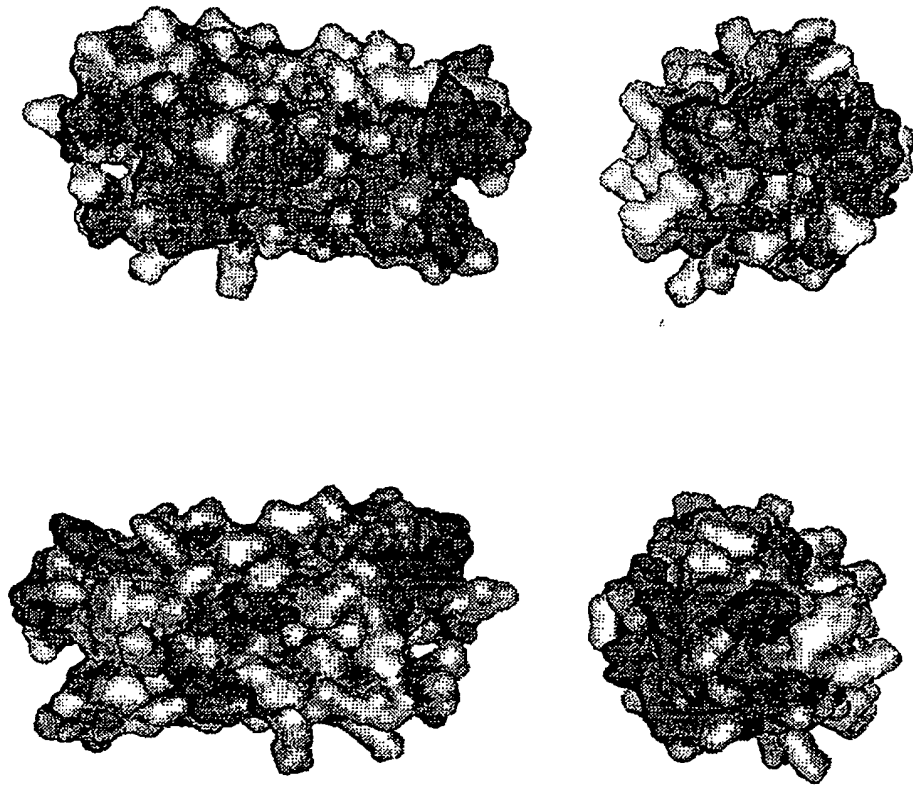


FIG. 39A: Phl p 5, Model A

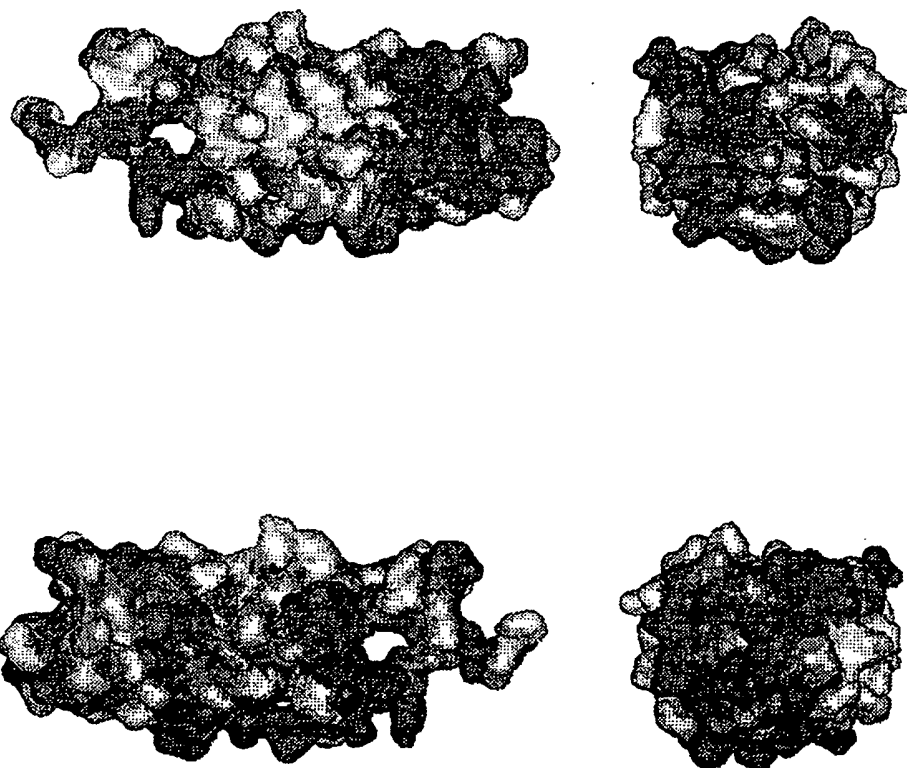


FIG. 39B: Phl p 5, Model B

10001245.111504

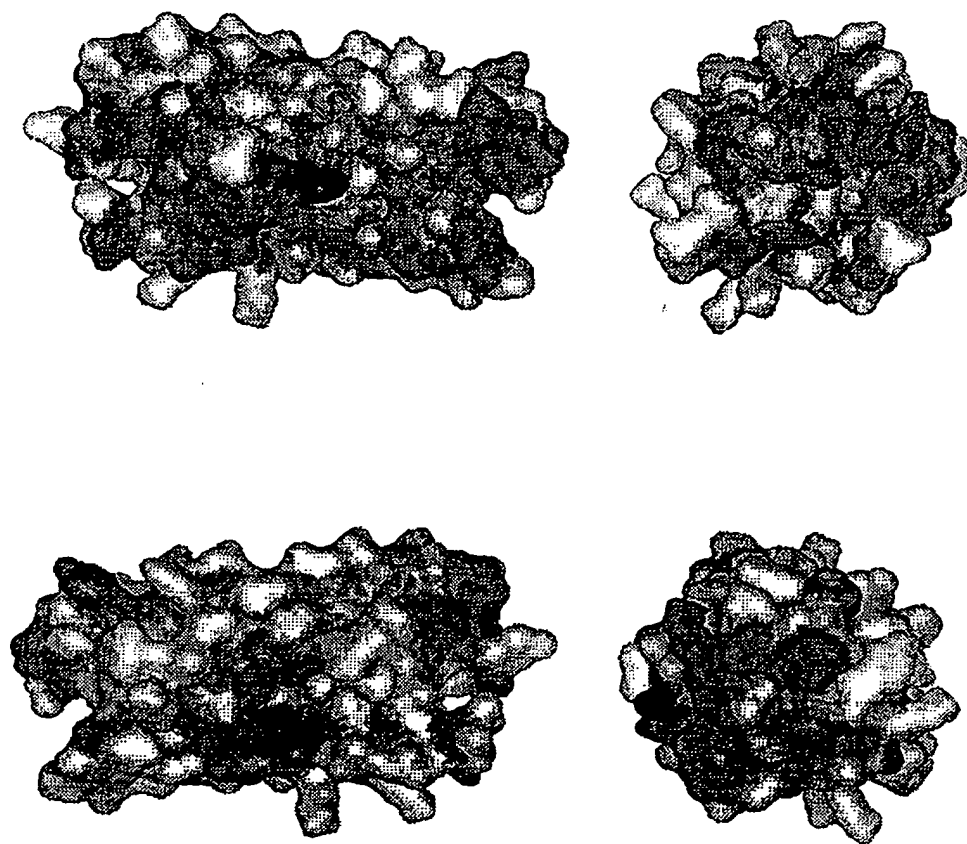


FIG. 40A: Phl p 5 mutant, Model A



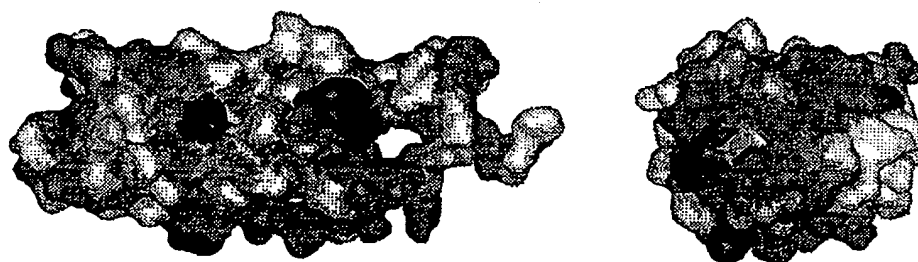
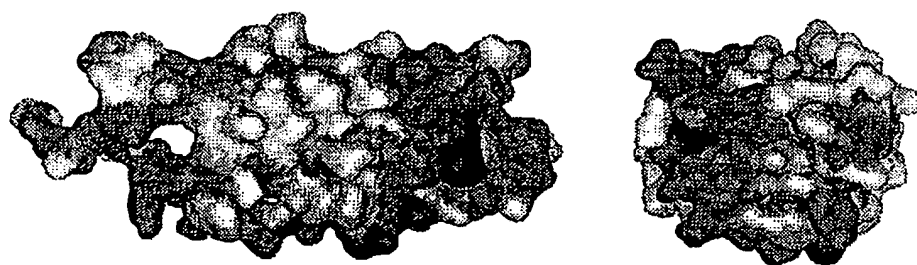


FIG. 40B: Phl p 5 mutant, Model B

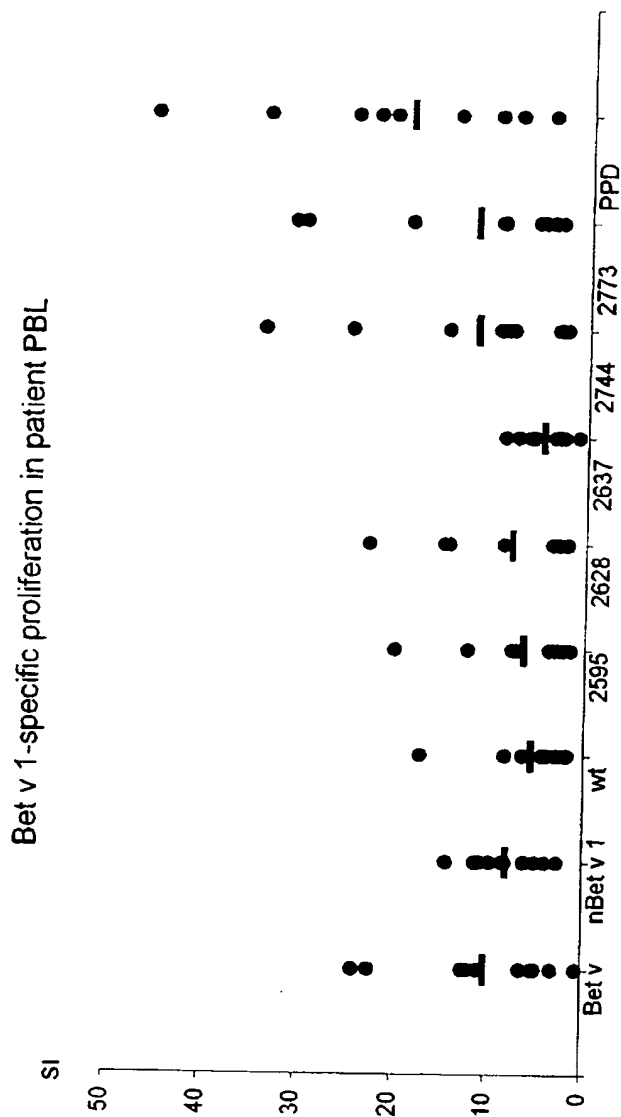
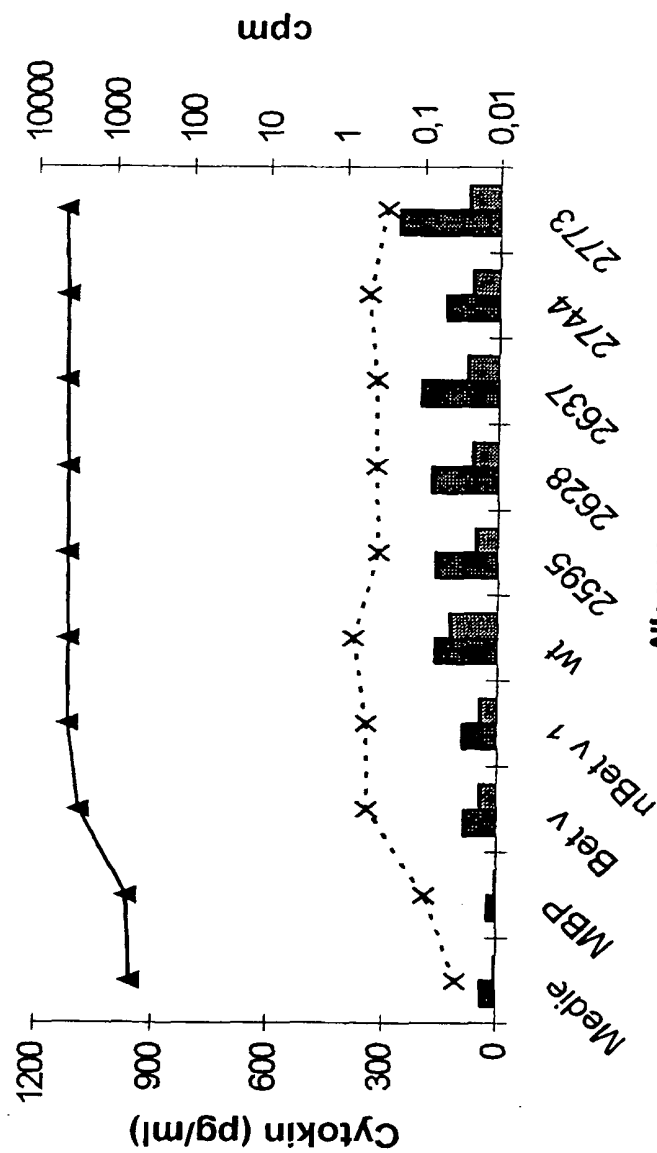
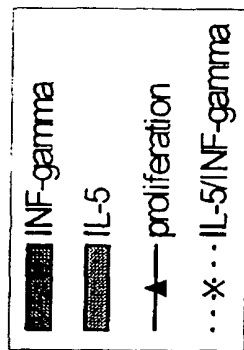


Figure 41: Stimulation of Bet v 1 samples



Allergen

FIG. 42

1500 1000 500 0

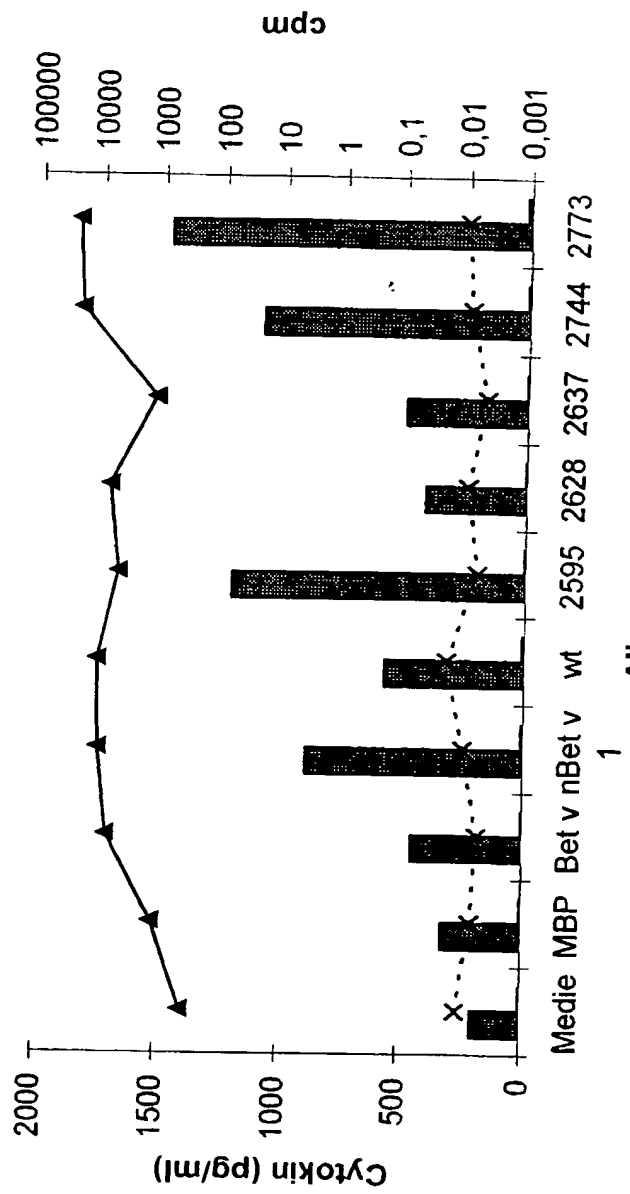
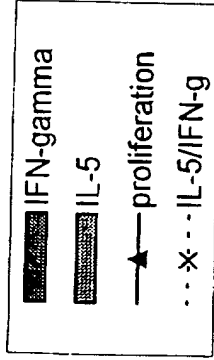


FIG. 43

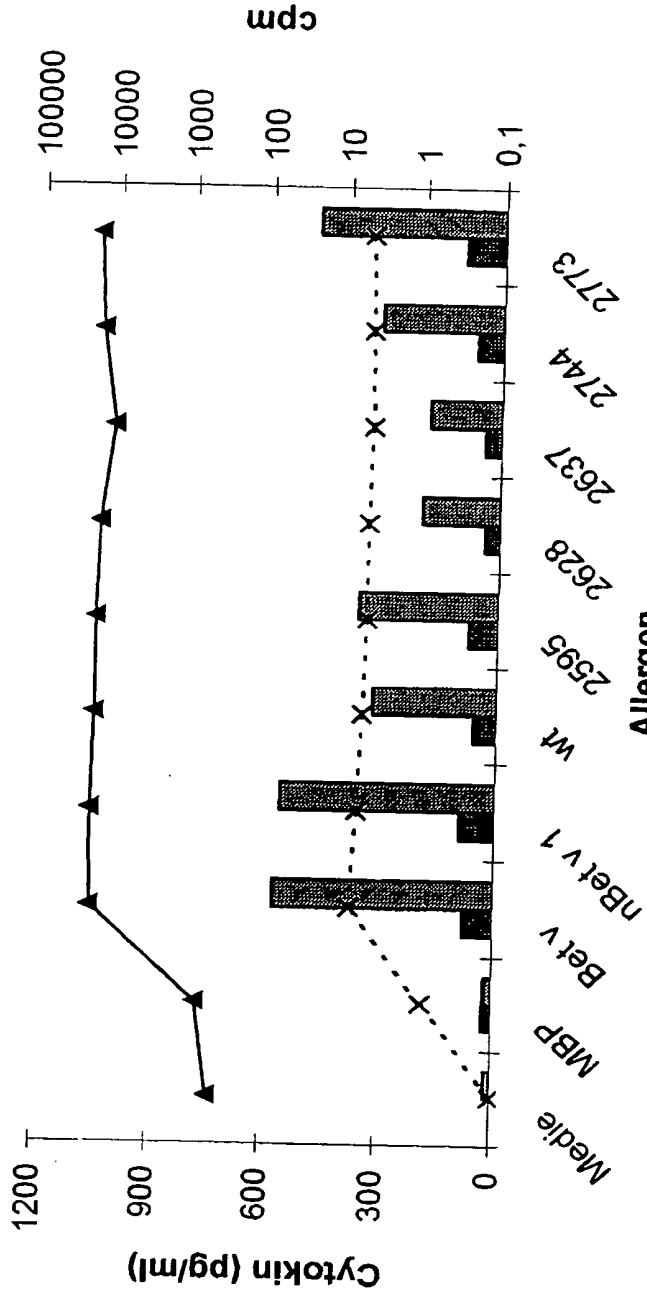
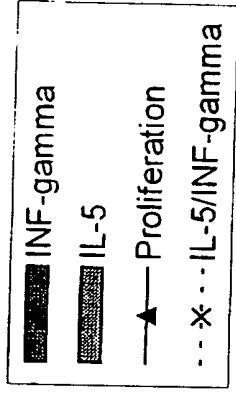


FIG. 44